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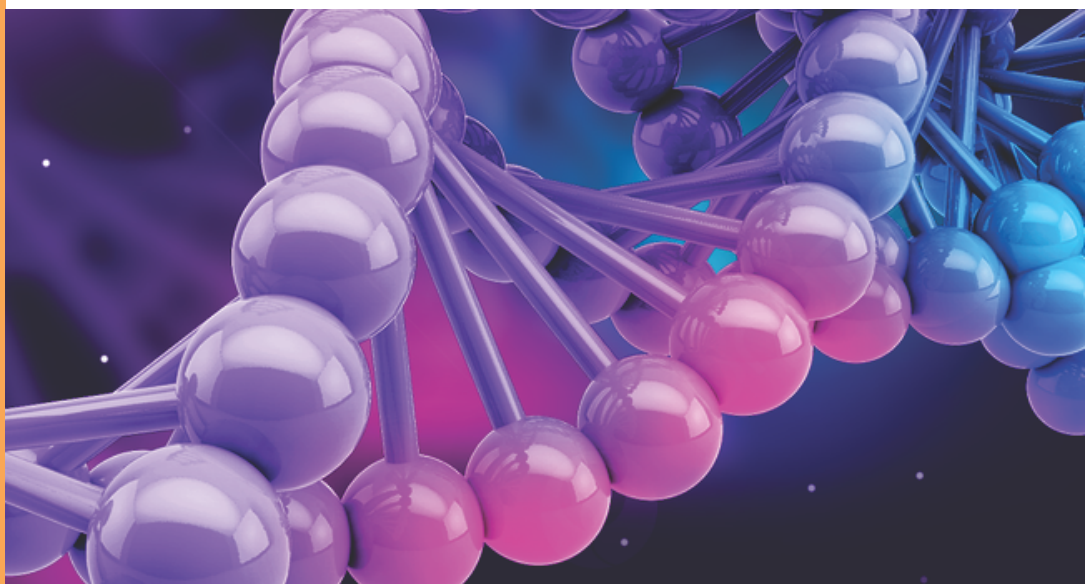
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- To promote and undertake research and development and extension service in the field of agriculture and biological science.
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Plant Regeneration Mechanisms in Tissue Culture: An In-Depth Analysis

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Abstract

Plant tissue culture represents a cutting-edge biotechnological approach, offering unparalleled capabilities for the *in vitro* manipulation and cultivation of plant cells, tissues, and organs. This sophisticated methodology is indispensable for a wide array of applications, significantly contributing to the rapid and efficient propagation of valuable plant species, their genetic improvement through various biotechnological interventions, and the vital conservation of biodiversity, particularly for endangered or economically important germplasm. At the core of the successful application of plant tissue culture lies the intricate understanding and precise manipulation of inherent plant regeneration mechanisms. These mechanisms define the capacity of isolated plant cells or tissues to dedifferentiate and subsequently redifferentiate, forming organized structures that can develop into complete, fertile plants. This comprehensive write-up specifically delves into the primary and most significant pathways of plant regeneration observed *in vitro*: organogenesis and somatic embryogenesis. Organogenesis, which can proceed either directly from the explant or indirectly via an intermediate callus phase, involves the formation of adventitious shoots and roots. In contrast, somatic embryogenesis leads to the development of bipolar embryonic structures directly from somatic cells, mimicking zygotic development. Gaining a profound understanding of these intricate cellular and molecular mechanisms, which are largely governed by the remarkable plasticity of plant cells and their dynamic response to carefully controlled nutritional and hormonal environments, is absolutely paramount. Such knowledge is critical not only for optimizing existing tissue culture protocols but also for driving future innovations in agricultural productivity, horticultural efficiency, and the broader advancement of plant biotechnology.

Keywords: Plant Tissue Culture, Regeneration, Organogenesis, Somatic Embryogenesis, Totipotency, Plant Growth Regulators.

Introduction

Plant tissue culture represents a cornerstone of modern plant biotechnology, offering a powerful suite of techniques for manipulating plant cells, tissues, and organs under controlled laboratory conditions. At its core, plant tissue culture involves the *in vitro* aseptic cultivation of plant cells, tissues, or organs on a nutrient medium

under controlled nutritional and environmental conditions (Murashige, 1974). This approach leverages the remarkable inherent plasticity of plant cells, allowing for their regeneration into entire, fully functional plants, often in situations where conventional propagation methods are inefficient or impossible. The historical roots of this field trace back to the early 20th century, notably with Gottlieb Haberlandt's pioneering concept of *in vitro* cell culture in 1902, where he theorized the totipotency of plant cells—their ability to develop into a whole plant. Although he was unable to achieve this himself, his visionary ideas laid the theoretical groundwork. Decades

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later, a significant breakthrough occurred with the discovery of plant hormones, auxins and cytokinins, and their synergistic interaction in regulating cell division and differentiation, elucidated by Skoog and Miller (1957). This fundamental understanding of plant growth regulators paved the way for successful *in vitro* cultivation and regeneration protocols.

The significance of plant tissue culture extends across numerous scientific and commercial applications, revolutionizing various sectors of agriculture, horticulture, and plant science. One of its most impactful applications is micropropagation, a rapid and efficient method for clonal propagation of plants, allowing for the mass production of genetically identical individuals from a small piece of parent material. This is particularly valuable for species that are difficult to propagate conventionally or for maintaining elite lines. Furthermore, plant tissue culture plays a crucial role in disease elimination, especially in producing virus-free plants. By culturing meristematic tissues, which are often free of viruses even in infected parent plants, healthy plantlets can be regenerated, greatly improving crop yield and quality. The techniques are also indispensable in genetic transformation, providing a system for introducing foreign genes into plant cells and subsequently regenerating genetically modified plants, which is vital for enhancing traits like pest resistance or nutritional value. In the realm of germplasm conservation, *in vitro* storage of plant tissues, seeds, or embryos offers a compact and secure method for preserving genetic diversity, especially for endangered species or valuable crop varieties. Beyond propagation and genetic improvement, tissue culture enables the production of secondary metabolites by culturing plant cells in bioreactors, offering a sustainable source of high-value compounds used in pharmaceuticals, cosmetics, and food industries. Lastly, it serves as an invaluable tool for basic research on plant development, allowing scientists to study cellular differentiation, hormonal responses, and genetic control of growth in a controlled environment, free from environmental variability.

Given the broad utility and foundational principles of plant tissue culture, this write-up will specifically focus on elucidating the fundamental mechanisms by which whole plants regenerate from cultured tissues. It will delve into the various pathways of regeneration, the critical factors influencing these processes, and the underlying molecular and genetic controls that govern plant totipotency and differentiation *in vitro*.

Fundamental Principles of Plant Regeneration

The remarkable capacity of plants to regenerate complete organisms from mere fragments of tissue or even single cells under *in vitro* conditions is underpinned by several fundamental biological principles. Understanding these principles is crucial for mastering plant tissue culture techniques and manipulating plant development.

Totipotency

At the heart of plant regeneration is the concept of totipotency, defined as the ability of a single plant cell to divide and differentiate into all the cell types necessary to form a complete, fertile plant (Steward *et al.*, 1958). Unlike most animal cells, mature plant somatic cells generally retain their full genetic potential (genome constancy), meaning they possess all the necessary genetic information to direct the development of a whole organism. This inherent plasticity allows botanists and biotechnologists to induce plant cells, typically from specialized tissues, to revert to an undifferentiated state and subsequently redifferentiate into new organs or even an entire plant. In *in vitro* culture, totipotency is expressed and modulated through the careful manipulation of the culture environment, particularly the composition of the nutrient medium and the balance of plant growth regulators. The cellular environment provided in tissue culture effectively unlocks this dormant genetic potential, enabling cells to embark on new developmental pathways.

Dedifferentiation and Redifferentiation

The process of *in vitro* regeneration typically involves two key cellular transitions:



dedifferentiation and redifferentiation. Dedifferentiation is the initial phase where specialized, mature plant cells revert to a meristematic or juvenile state, characterized by active cell division and a less specialized morphology (Feher, 2019). This involves a reprogramming of gene expression patterns, allowing these cells to lose their previous identity and become more plastic. Following dedifferentiation, redifferentiation occurs, wherein these now totipotent or pluripotent cells develop into new, organized structures such as shoots, roots, or even embryos. This phase involves a coordinated process of cell division, expansion, and differentiation into various tissues and organs, ultimately leading to the formation of a whole plant. These two processes highlight the dynamic nature of plant cell fate and their capacity for developmental plasticity under specific environmental cues.

Role of Plant Growth Regulators (PGRs)

Plant growth regulators (PGRs), often referred to as plant hormones, are chemical messengers that profoundly influence plant growth and development at very low concentrations. In plant tissue culture, the precise balance and concentration of exogenous PGRs in the culture medium are paramount for dictating the regeneration pathway.

- **Auxins:** These PGRs are primarily known for promoting cell elongation, apical dominance, and root development. In tissue culture, auxins like Indole-3-acetic acid (IAA), Naphthaleneacetic acid (NAA), and 2,4-Dichlorophenoxyacetic acid (2,4-D) are critical for inducing cell division, initiating callus formation, and stimulating root differentiation (Gaspar *et al.*, 1996). 2,4-D, in particular, is a strong synthetic auxin often used for callus induction and somatic embryogenesis due to its potent and persistent effects.
- **Cytokinins:** Cytokinins are essential for promoting cell division, delaying senescence, and stimulating shoot proliferation. Common cytokinins used in tissue culture include Kinetin, 6-Benzylaminopurine (BAP), and

Zeatin. They play a vital role in overcoming apical dominance and promoting axillary bud proliferation, leading to multiple shoot formation (Skoog and Miller, 1957).

- **Auxin-to-Cytokinin Ratio:** The relative concentrations of auxins and cytokinins are perhaps the most critical factor in determining the developmental fate of cultured plant tissues (Skoog and Miller, 1957). A high auxin-to-cytokinin ratio typically favors root formation, while a high cytokinin-to-auxin ratio promotes shoot differentiation. A balanced ratio of both hormones often leads to the proliferation of undifferentiated callus tissue. This intricate hormonal interplay directs the pathways of organogenesis and somatic embryogenesis.
- **Gibberellins and Absciscic Acid (ABA):** While auxins and cytokinins are the primary regulators, other PGRs also play secondary, yet significant, roles. Gibberellins (GAs) can promote shoot elongation and germination, but sometimes inhibit callus growth or adventitious shoot formation. Absciscic acid (ABA) is crucial in inducing and regulating embryo development, particularly during the maturation phase of somatic embryogenesis, enhancing desiccation tolerance and promoting storage protein accumulation in somatic embryos.

Culture Medium Components

Beyond PGRs, the complete formulation of the culture medium is fundamental for successful *in vitro* growth and regeneration. A typical plant tissue culture medium, such as the widely used Murashige and Skoog (MS) medium, comprises a complex blend of essential nutrients (Murashige and Skoog, 1962). These include macronutrients (e.g., nitrogen, phosphorus, potassium, calcium, magnesium, sulfur) required in larger quantities, and micronutrients (e.g., iron, manganese, zinc, boron, copper, molybdenum, cobalt, iodine) needed in trace amounts. Additionally, vitamins (e.g., thiamine, nicotinic acid, pyridoxine), which serve as coenzymes in metabolic pathways, and a carbohydrate source, most commonly sucrose, are vital. Sucrose provides the carbon and energy



necessary for heterotrophic growth of the cultured tissues. Finally, a gelling agent, such as agar, is often included to solidify the medium, providing physical support for the explant and developing plantlets. The precise balance and availability of all these components are crucial for optimal cell growth, differentiation, and the successful regeneration of whole plants.

Mechanisms of Plant Regeneration

Plant regeneration *in vitro* primarily occurs through two major pathways: organogenesis and somatic embryogenesis. Both pathways involve the fundamental principles of totipotency, dedifferentiation, and redifferentiation, but they differ significantly in their developmental patterns and the type of structures formed.

Organogenesis (Formation of Organs)

Organogenesis is the process by which adventitious organs (shoots or roots) develop from cultured plant tissues. This pathway can occur either directly from the explant or indirectly via an intermediate callus phase. The initiation and development of these organs are meticulously controlled by the interaction of plant growth regulators, the physiological state of the explant, and various environmental cues.

1. Direct Organogenesis

Direct organogenesis involves the formation of shoots or roots directly from the explant tissue without the development of an intervening, unorganized callus phase. This pathway is characterized by a direct transition of specialized cells within the explant to form new meristematic centers, which then differentiate into organized structures. The mechanism often involves the activation of pre-existing meristematic cells or the rapid induction of meristematic activity within the explant's somatic cells, leading directly to organ primordia formation (George and Sherrington, 1984). For instance, adventitious shoots can directly emerge from nodal segments, or roots can form directly from stem cuttings placed in an appropriate rooting medium.

The primary advantages of direct organogenesis include its speed, as it bypasses the often-time-

consuming callus phase, and a significantly reduced chance of somaclonal variation. Somaclonal variation refers to genetic and epigenetic changes that can accumulate during prolonged periods of unorganized cell proliferation (callus formation), leading to undesirable phenotypic alterations in the regenerated plants. Examples of direct organogenesis include the straightforward shoot regeneration from nodal segments of many plant species, or the direct formation of adventitious roots from the base of stem explants or microcuttings.

2. Indirect Organogenesis

In contrast, indirect organogenesis proceeds through an intermediate, unorganized mass of proliferating cells known as callus. In this pathway, the explant cells first undergo dedifferentiation to form callus tissue. This callus is an amorphous, undifferentiated mass of cells, which then proliferates. Subsequently, under specific hormonal and environmental conditions, certain cells within this callus mass redifferentiate, forming meristematic centers that develop into adventitious shoots or roots (Thorpe, 1995). The typical process involves: Explant → Callus induction → Callus proliferation → Shoot differentiation → Rooting.

Indirect organogenesis offers several advantages, including a potentially very high multiplication rate due to the rapid proliferation of callus. This makes it particularly useful for large-scale propagation. Furthermore, the callus phase is often a target for genetic transformation experiments, as the unorganized cells are more amenable to gene delivery methods, and the transformed cells can then be induced to regenerate whole plants. However, a significant disadvantage of indirect organogenesis is the higher risk of somaclonal variation. The extended period of unorganized growth and rapid cell division in the callus phase can lead to increased genetic instability, potentially resulting in variations among the regenerated plantlets.

3. Factors Influencing Organogenesis

The success and efficiency of both direct and



indirect organogenesis are profoundly influenced by a multitude of factors, necessitating careful optimization for each plant species and specific regeneration goal.

- **Explant Type and Physiological State:** The source tissue (explant) plays a critical role. Different explants, such as leaves, stems, roots, cotyledons, hypocotyls, or floral parts, possess varying capacities for regeneration. The developmental stage and physiological state of the donor plant from which the explant is taken also significantly impact regenerative capacity (Bhojwani and Razdan, 1996). Younger, actively growing tissues often exhibit a higher regenerative potential.
- **Genotype of the Plant:** Plant regeneration is highly genotype-dependent. Different species, and even different varieties within the same species, can exhibit vast differences in their responsiveness to tissue culture protocols. Some genotypes are highly recalcitrant and prove difficult to regenerate, while others respond readily.
- **PGR Concentrations and Balance:** As previously discussed, the precise balance between auxins and cytokinins is the most crucial chemical factor determining organogenesis. The specific type and concentration of these PGRs in the medium dictate whether callus will form, or whether shoots or roots will differentiate (Skoog and Miller, 1957). Optimized ratios are essential for inducing the desired developmental pathway.
- **Light, Temperature, and Humidity:** Environmental factors within the culture vessel and growth room are also important. Light quality, intensity, and photoperiod can influence chlorophyll development and morphogenetic responses. Temperature affects metabolic rates and cell division, with an optimal range typically between 22-28°C for most species. Humidity within the culture vessel is crucial, especially for the initial establishment and subsequent growth of plantlets.

Somatic Embryogenesis (Formation of Somatic Embryos)

Somatic embryogenesis is another powerful *in vitro* regeneration pathway, distinct from organogenesis, that allows for the development of bipolar structures resembling zygotic embryos directly from somatic (non-sexual) plant cells.

Somatic embryogenesis is the process by which a single somatic plant cell, or a group of cells, is induced to follow a developmental pathway that mimics the stages of zygotic embryogenesis, ultimately forming a somatic embryo that is capable of developing into a whole plant (Trewavas, 1982; Feheret *et al.*, 2003). Unlike zygotic embryos, which originate from the fusion of gametes (egg and sperm), somatic embryos originate from vegetative cells of the parent plant. These somatic embryos are characterized by their bipolar nature, possessing both a radicle (root pole) and a plumule (shoot pole) at opposite ends, and are enclosed by cotyledons, just like their zygotic counterparts.

Process of Somatic Embryogenesis

The induction and development of somatic embryos typically proceed through a series of distinct phases, although the specific requirements can vary greatly among species:

- **Induction Phase:** This initial phase is crucial for triggering embryogenic competence in somatic cells. It often requires the presence of a high concentration of auxins, particularly strong synthetic auxins like 2,4-D (2,4-Dichlorophenoxyacetic acid). The auxin promotes intense cell division and can induce the formation of embryogenic callus or individual cells that differentiate into suspensor-like structures, characteristic of early embryo development (Jimenez, 2005).
- **Developmental Phase:** Once embryogenic cells or callus are induced, they are typically transferred to a medium with reduced or no auxin, which allows for the progression of embryo development. During this phase, the somatic embryos pass through characteristic morphological stages analogous to those seen in zygotic embryogenesis: globular, heart,



torpedo, and cotyledonary stages (Jimenez, 2005). Each stage represents a specific phase of cellular organization and differentiation.

- **Maturation Phase:** This phase is vital for the proper physiological development of somatic embryos. It often involves the incorporation of abscisic acid (ABA) into the medium. ABA promotes crucial processes such as desiccation tolerance, accumulation of storage products (e.g., proteins, lipids, carbohydrates), and suppression of premature germination, preparing the embryo for ex vitro survival.
- **Germination Phase:** Mature somatic embryos are then transferred to a germination medium, typically with reduced or no PGRs, and under appropriate light and temperature conditions, to promote the development of a functional shoot and root system, leading to a complete plantlet.

Types of Somatic Embryogenesis

- Similar to organogenesis, somatic embryogenesis can be categorized into direct and indirect pathways:
- **Direct Somatic Embryogenesis:** In this pathway, somatic embryos form directly from the explant cells without the formation of an intervening callus phase. This direct initiation minimizes the risk of somaclonal variation and can lead to a more uniform population of embryos.
- **Indirect Somatic Embryogenesis:** This is the more common pathway, where somatic embryos develop from an embryogenic callus. The callus acts as an intermediate proliferative stage, from which discrete embryogenic cells or cell clusters differentiate into somatic embryos. While this offers a high multiplication rate, it carries a greater risk of somaclonal variation due to the callus phase.

Advantages of Somatic Embryogenesis

Somatic embryogenesis holds several significant advantages, making it a highly attractive pathway for various biotechnological applications:

- **Potential for Mass Propagation in Bioreactors:** The ability to produce numerous individual

embryos that are structurally and physiologically similar allows for scalable propagation in liquid culture systems, such as bioreactors, which can automate and industrialize plant production.

- **Artificial Seed Production:** Somatic embryos can be encapsulated in a protective matrix (e.g., calcium alginate) to create "artificial seeds" or "synthetic seeds". These artificial seeds can be handled and sown like conventional seeds, offering a potential means for germplasm dissemination and planting.
- **Applicable for Genetic Transformation:** The single-cell or small cluster origin of somatic embryos, particularly from suspension cultures, makes them highly amenable targets for genetic transformation techniques, as individual transformed cells can be selected and regenerated.
- **Conservation of Endangered Species:** For species with recalcitrant seeds or those facing extinction, somatic embryogenesis provides an effective alternative for rapid multiplication and long-term conservation of valuable germplasm.

Factors Influencing Somatic Embryogenesis

- The successful induction and development of somatic embryos are influenced by a complex interplay of genetic and environmental factors:
- **Explant Type and Developmental Stage:** The choice of explant (e.g., immature embryos, cotyledons, hypocotyls, leaves) and its precise developmental stage are critical determinants of embryogenic competence. Younger tissues often show higher responsiveness.
- **Auxin Type and Concentration During Induction:** The type and concentration of auxin used during the induction phase are paramount. 2,4-D is frequently preferred for its strong embryogenesis-inducing activity (Jimenez, 2005). Optimal concentrations vary widely depending on the plant species.
- **Nitrogen Source and Concentration:** The form and concentration of nitrogen (e.g., ammonium to nitrate ratio) in the culture medium can



significantly impact somatic embryogenesis, affecting cell division and embryo maturation.

- **Genotype:** As with organogenesis, genotype plays a dominant role. Some plant species and genotypes are highly embryogenic, while others are recalcitrant.
- **Stress Factors:** Interestingly, certain abiotic stress factors, such as heat shock, desiccation, or osmotic stress (e.g., high sugar concentration), can sometimes act as elicitors, promoting or enhancing the frequency of somatic embryogenesis in some species.

Molecular and Genetic Basis of Regeneration

Beyond the macroscopic observations of *in vitro* regeneration, understanding the intricate molecular and genetic mechanisms that underpin these processes is crucial. Plant regeneration involves complex gene regulatory networks and signalling pathways that control cell fate, division, and differentiation.

Role of Key Genes

The ability of plant cells to dedifferentiate and then redifferentiate into organized structures is governed by the precise spatio-temporal expression of a suite of developmental genes:

- **WUSCHEL (WUS) and SHOOT MERISTEMLESS (STM):** These are homeodomain transcription factors that play central roles in maintaining stem cell identity and organizing the shoot apical meristem (SAM). *WUS* acts in the organizing center of the SAM to promote stem cell fate, while *STM* is essential for SAM initiation and maintenance (Brand *et al.*, 2000; Long *et al.*, 1996). Their coordinated expression is critical during the *de novo* formation of shoots in organogenesis, guiding the assembly of a functional meristem.
- **LEAFY COTYLEDON (LEC), FUSCA3 (FUS3), and AGL15:** These genes, often referred to as "LAFL" genes, are key regulators of embryo development and maturation in both zygotic and somatic embryogenesis. They are essential for promoting cell proliferation, establishing embryo identity, and facilitating the

accumulation of storage reserves (Braybrook and Sussex, 2008). Their sustained expression is crucial for the successful progression of somatic embryos through their developmental stages.

- **PLETHORA (PLT) genes:** These AP2-domain transcription factors are fundamental for establishing and maintaining the root stem cell niche. They are involved in various aspects of root development, including stem cell activity and patterning (Aida *et al.*, 2004). Their activation is therefore crucial for the proper formation of roots during organogenesis.

Hormonal Signal Transduction Pathways

The perception of plant growth regulators by cultured cells triggers complex intracellular signal transduction pathways that ultimately lead to changes in gene expression, driving cell division and differentiation.

- **Auxin Signaling:** Auxins are perceived by TIR1/AFB F-box proteins, which act as co-receptors with Aux/IAA proteins. This perception leads to the degradation of Aux/IAA repressors, allowing Auxin Response Factors (ARFs) to activate or repress target genes involved in cell elongation, division, and patterning (DelloIoio *et al.*, 2008). In tissue culture, the specific auxin concentration dictates which ARFs are active, thereby channelling developmental responses towards callus formation, root initiation, or even embryogenesis.
- **Cytokinin Signaling:** Cytokinins are perceived by histidine kinase receptors located at the plasma membrane. This triggers a phosphorelay system, ultimately leading to the phosphorylation of B-type ARRs (Arabidopsis Response Regulators), which then act as transcription factors to regulate the expression of cytokinin-responsive genes. These genes are involved in cell proliferation, shoot regeneration, and meristem activity (Kieber and Schaller, 2014). The interplay between auxin and cytokinin signalling pathways is a central regulatory hub that dictates the *in vitro* morphogenetic outcome.



Epigenetic Modifications

Beyond the primary DNA sequence, epigenetic modifications play a significant role in regulating gene expression during the dynamic processes of dedifferentiation and redifferentiation in plant tissue culture. These heritable changes, which do not involve alterations to the underlying DNA sequence, include DNA methylation and histone modifications. DNA methylation, often occurring at cytosine residues, can lead to gene silencing when present in promoter regions. Histone modifications (e.g., acetylation, methylation, phosphorylation) alter chromatin structure, making genes more or less accessible for transcription. The complex interplay of these epigenetic marks facilitates the necessary gene reprogramming that allows specialized somatic cells to revert to a totipotent state (dedifferentiation) and subsequently redifferentiate into new organs or embryos (Feher, 2019). Research in this emerging area is shedding light on how plant cells achieve their remarkable plasticity and how it can be harnessed for improved regeneration protocols.

Challenges and Future Perspectives

Despite the significant advancements in plant tissue culture, several challenges persist that limit its widespread application and efficiency across all plant species. Addressing these challenges is crucial for unlocking the full potential of *in vitro* regeneration for agricultural, horticultural, and conservation purposes.

Challenges

- **Genotype Dependency (Recalcitrance):** One of the most significant challenges in plant tissue culture is the high degree of genotype dependency. The ability to regenerate a whole plant from cultured tissues varies enormously between different plant species and even among different cultivars within the same species. Many economically important crops and forest trees are recalcitrant, meaning they are difficult or impossible to regenerate using current *in vitro* protocols. This recalcitrance often stems from complex genetic factors that are not yet fully understood, limiting the application of tissue culture for these valuable genotypes.
- **Somaclonal Variation:** While *in vitro* propagation aims to produce genetically identical copies, somaclonal variation—the occurrence of genetic and epigenetic changes in plants regenerated from tissue culture—remains a major concern. This phenomenon is particularly prevalent in indirect regeneration pathways involving a prolonged callus phase, where rapid, unorganized cell divisions can lead to increased mutation rates or epigenetic instability. These variations can result in undesirable phenotypic changes in the regenerated plants, affecting traits like yield, disease resistance, or quality.
- **Vitrification (Hyperhydricity):** Vitrification, also known as hyperhydricity, is a physiological disorder commonly observed in *in-vitro* cultured plants, particularly under high humidity and inadequate ventilation. Affected plantlets appear translucent, water-soaked, and glassy, with brittle leaves and stems. This condition is characterized by abnormal water uptake, poor cuticular development, and impaired stomatal function, leading to a reduced survival rate upon transfer to *ex vitro* conditions.
- **Contamination:** Maintaining aseptic conditions is paramount in plant tissue culture. Contamination by bacteria, fungi, yeasts, or even other plant tissues can quickly ruin entire cultures, leading to significant material and time losses. Sources of contamination can include the explant itself, inadequately sterilized media, instruments, or the laboratory environment, requiring rigorous sterile techniques and vigilant monitoring.
- **Cost-effectiveness for Large-Scale Production:** While highly effective for small-scale propagation, the cost-effectiveness of traditional plant tissue culture methods for large-scale commercial production remains a challenge. The labor-intensive nature of manual handling, the need for sterile environments, and the cost of specialized media and equipment can make *in vitro* propagation more expensive than



conventional methods for certain crops. This limits its economic viability for many agricultural applications.

Future Perspectives

Despite these challenges, the field of plant tissue culture continues to evolve rapidly, driven by advancements in molecular biology and biotechnology. Future perspectives are focused on leveraging these new insights and technologies to overcome existing limitations and broaden the applications of *in vitro* regeneration.

- **Deepening Understanding of Molecular Mechanisms:** A primary future direction involves further deepening the understanding of the molecular and genetic mechanisms underlying plant regeneration. By elucidating the precise gene networks, signaling pathways, and transcriptional regulators involved in totipotency, dedifferentiation, and redifferentiation, researchers aim to develop more universal and efficient regeneration protocols that are less genotype-dependent.
- **Application of '-omics' Technologies:** The integration of '-omics' technologies, including genomics, transcriptomics, proteomics, and metabolomics, is pivotal. These high-throughput approaches allow for the comprehensive identification of key genes, proteins, and metabolites that regulate various stages of *in vitro* regeneration. This data can provide molecular markers for embryogenic competence, inform the design of optimized media, and help pinpoint the causes of recalcitrance in challenging species.
- **CRISPR/Cas9 for Targeted Modification of Regeneration-Related Genes:** The advent of precise genome editing tools like CRISPR/Cas9 offers unprecedented opportunities for targeted modification of regeneration-related genes. By precisely altering the expression or function of genes known to influence totipotency or meristem development, it may be possible to enhance the regenerative capacity of recalcitrant species or to engineer plants with improved *in vitro* responses.
- **Automation and Bioreactor Technology for**

Large-Scale Production: To address the issue of cost-effectiveness, significant efforts are being directed towards automation and the widespread adoption of bioreactor technology for large-scale *in vitro* plant propagation. Bioreactor systems offer a controlled environment for liquid culture, reducing labor, optimizing nutrient delivery, and facilitating higher biomass production and regeneration rates, thereby making micropropagation more economically viable for commercial applications.

- **Exploration of Novel Plant Growth Regulators or Elicitors:** Research will continue to explore novel plant growth regulators or elicitors that can enhance regeneration efficiency, reduce somaclonal variation, or induce regeneration in difficult-to-culture species. This includes investigating synthetic compounds, natural plant extracts, or even microbial elicitors that can fine-tune developmental pathways *in vitro*.

Conclusion

Plant regeneration mechanisms in tissue culture are fundamental to modern plant biotechnology, harnessing the inherent totipotency of plant cells to produce whole plants from cultured tissues. The primary pathways, organogenesis (direct and indirect) and somatic embryogenesis, each offer unique advantages and are meticulously controlled by a complex interplay of plant growth regulators, medium composition, environmental conditions, and underlying genetic programs. While organogenesis focuses on the development of adventitious shoots and roots, somatic embryogenesis leads to the formation of bipolar embryonic structures, each capable of developing into a complete plant. The intricate molecular and genetic basis, involving key developmental genes and hormonal signaling pathways, provides the framework for these remarkable regenerative capacities. Despite ongoing challenges such as genotype dependency, somaclonal variation, and the need for cost-effective large-scale production, continuous advancements in molecular biology, genomics, and automation are paving the way for more efficient, precise, and broadly applicable *in*

vitro regeneration protocols. Understanding and optimizing these mechanisms are paramount for their continued application in agricultural improvement, germplasm conservation, and biotechnological innovations.

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Zinc Nanoparticle Enhanced Phytoremediation of Contaminated Soil Using *Brassica juncea*

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Abstract

This study investigates the potential of *Brassica juncea* (Indian mustard) enhanced with green-synthesized zinc nanoparticles (ZnNPs) for phytoremediation of soils contaminated with heavy metals, specifically lead (Pb) and copper (Cu). ZnNPs were synthesized using *Trigonella foenum-graecum* (fenugreek) leaf extract through an eco-friendly, cost-effective method and characterized using UV-Visible spectroscopy, FTIR and SEM, confirming their nanoscale size and stability. A pot experiment was conducted with four treatment groups: untreated soil (NM), heavy metal-contaminated soil (HM), HM soil treated with ZnNPs (T1) and HM soil treated with commercial zinc fertilizer (T2). Results indicated that ZnNP treatment (T1) significantly improved germination, plant height, leaf number, chlorophyll content and reproductive traits while also enhancing Pb and Cu uptake, outperforming both HM and T2 treatments. The findings demonstrate that ZnNPs boost both plant growth and phytoextraction efficiency under metal stress, likely by enhancing antioxidant activity and metal bioavailability. This study presents nano-phytoremediation using ZnNPs and *B. juncea* as a promising, sustainable strategy for remediating metal-polluted soils. Future research should explore field applications, optimize nanoparticle dosage and assess long-term ecological impacts.

Keywords: Phytoremediation, zinc nanoparticles, *Brassica juncea*, heavy metal uptake, green synthesis, soil remediation.

Introduction

Brassica juncea, commonly known as mustard, is a versatile cruciferous vegetable from the Brassicaceae family, valued for its pungent flavor, nutritional benefits and historical use in traditional medicine. Rich in vitamins, minerals, dietary fiber, glucosinolates, polyphenols and volatile compounds, its composition varies by variety, environment and processing methods (Tian and Deng, 2020). Beyond its culinary uses, *B. juncea* exhibits antioxidant, anti-inflammatory and antimicrobial properties, showing potential in managing diseases like cancer, obesity and diabetes (Tian and Deng, 2020). Despite growing

research on cruciferous vegetables, comprehensive studies specifically on *B. juncea*'s health benefits are still needed (Cartea *et al.*, 2008). The *Brassica* genus, including *B. juncea*, is globally significant for its contributions to food, fodder, oilseed production and potential as a biofuel. *B. juncea*, an amphidiploid hybrid ($2n = 36$, AABB) derived from *B. nigra* and *B. rapa*, is a major edible oilseed crop in countries like India, Pakistan, Bangladesh and China. Its adaptability to cooler climates and ability to germinate at low temperatures make it ideal for temperate regions, high altitudes and winter cropping (Rani *et al.*, 2023). The plant thrives in well-drained, fertile soils with a pH of 6.0–7.5 and has a short growth cycle of 90–120 days, enabling quick harvests. Its seeds are a source of mustard oil, high in unsaturated fatty acids and are used in food and animal feed due to their protein content. Soil contamination, particularly by heavy metals like lead (Pb) and copper (Cu), poses significant

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risks to agriculture, ecosystems and human health. Metals like lead, cadmium, mercury and arsenic accumulate in organisms, causing oxidative stress by generating reactive oxygen species (ROS), which can damage DNA, proteins and lipids, leading to chronic diseases (Valco *et al.*, 2005). Lead, found in paints, batteries and industrial waste, is highly toxic and prevalent at over half of U.S. National Priorities List sites (ATSDR, 2022). Copper, while essential in trace amounts, becomes toxic at high levels due to industrial and agricultural activities, affecting aquatic and terrestrial ecosystems.

Phytoremediation uses plants and soil microbes to reduce pollutant toxicity, offering a cost-effective, eco-friendly alternative to traditional remediation methods. Techniques include phytoextraction (absorbing metals into plant tissues), phytostabilization (immobilizing metals in soil) and phytovolatilization (releasing less toxic forms into the air) (Pilon-Smits, 2005). *B. juncea* excels in phytoextraction, efficiently absorbing metals like lead, cadmium and chromium due to its high biomass, rapid growth and robust root system (Ali *et al.*, 2013). It employs mechanisms like metal sequestration in vacuoles, binding to cell walls and antioxidant enzyme activity (e.g., superoxide dismutase, catalase) to tolerate metal stress (Hall, 2002; Sharma and Dubey, 2005).

Nanotechnology enhances phytoremediation through nano-phytoremediation, where nanoparticles (NPs) improve metal uptake and plant tolerance. NPs, sized 1–100 nm, interact with plant tissues, enhancing nutrient delivery, stress tolerance and metal sequestration (Ojuederie *et al.*, 2022). Zinc oxide nanoparticles (ZnO NPs) are particularly promising, promoting plant growth, root elongation and antioxidant activity, thus aiding metal detoxification (Duhan *et al.*, 2017). Zn NPs improve zinc bioavailability, addressing deficiencies in crops like wheat and maize and enhance stress resistance by modulating enzymes like Superoxide dismutase (SOD) and Catalase (CAT) (Sheteiwy *et al.*, 2021; Faizan *et al.*, 2021).

B. juncea absorb metals through root transporters,

binding them to cell walls or sequestering them in vacuoles via phytochelatins and metallothioneins (Clemens, 2006; Martinoia *et al.*, 2007). Organic acids like citrate and malate reduce metal toxicity by chelating ions in the rhizosphere. Redox enzymes can transform toxic metals (e.g., Cr^{6+} to Cr^{3+}), further aiding detoxification (Krämer, 2010). NPs enhance these processes by improving root architecture, metal-binding peptide production and rhizosphere chemistry (Ojuederie *et al.*, 2022).

This study's focus on combining *B. juncea* with Zn NPs addresses soil contamination in regions like India, where heavy metals threaten agriculture and food security. Nano-phytoremediation offers a sustainable, cost-effective solution, enhancing crop resilience and soil health (Ali *et al.*, 2013). However, gaps remain: limited research on Zn NPs' role in phytoremediation, optimal NP concentrations, mechanisms of NP-plant interactions, long-term environmental impacts and field-scale applications. Addressing these gaps could advance nano-phytoremediation, offering scalable solutions for contaminated soils and sustainable agriculture.

Methodology

The methodology for preparing plant extract and synthesizing ZnNPs involved a green synthesis approach using fenugreek (*Trigonella foenum-graecum*) leaf extract to reduce zinc ions from zinc sulphate heptahydrate ($\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$) into nanoparticles, with phytochemicals acting as reducing and stabilizing agents. Fresh fenugreek leaves were cleaned, air-dried, ground into powder and boiled in distilled water to obtain the extract, which was filtered and stored at 4°C. A 0.1 M zinc sulphate solution was prepared by dissolving 2.88 g of $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ in 500 mL of distilled water. For nanoparticle synthesis, 400 mL of this solution was mixed with 100 mL of fenugreek extract, stirred at 70°C and monitored for a color change from light yellow to pale brown, indicating ZnNP formation, confirmed by a control setup with distilled water showing no change. The synthesized ZnNPs were characterized using UV-Visible



spectrophotometry to detect surface plasmon resonance (SPR), Fourier Transform Infrared (FTIR) spectroscopy to identifying functional groups and Scanning Electron Microscopy (SEM) to analyse nanoparticles' morphology. Soil experiments involved collecting non-contaminated soil, artificially contaminating it with 50 mg/kg of lead acetate and cupric sulphate and distributing it into 20 pots across four treatment groups: control or normal untreated soil (NM), heavy metal-contaminated soil (HM), contaminated soil with ZnNPs (T1) and contaminated soil with commercial zinc fertilizer (T2), each with five replicates. *Brassica juncea* seeds were surface-sterilized and sown in pots under controlled greenhouse conditions. Treatments were applied every 15 days, with T1 using a sonicated ZnNP solution and T2 using a 0.5 g/500 mL zinc EDTA fertilizer solution. After 45 days, plant growth parameters (germination, flowering, height, leaf count, leaf size, siliquae and seed count) were recorded and chlorophyll content was measured non-destructively using a SPAD-502 Plus meter. Heavy metal uptake (Pb and Cu) was quantified via Atomic Absorption Spectrophotometry (AAS) after tri-acid digestion of plant tissues. Statistical analysis using one-way ANOVA in SPSS compared treatment effects to determine the efficacy of ZnNPs in enhancing phytoremediation and plant growth under heavy metal stress.

Results and Discussion

The green synthesis of zinc nanoparticles (ZnNPs) was conducted using an aqueous extract derived from dry leaves of fenugreek (*Trigonella foenum-graecum*), offering an eco-friendly, cost-effective and straightforward approach to nanoparticle production. The synthesis involved mixing a zinc sulfate (ZnSO_4) precursor solution with the fenugreek leaf extract, which contains bioactive compounds such as flavonoids, saponins, alkaloids and polyphenols (fig. 1). These phytochemicals served dual roles as reducing agents, converting zinc ions (Zn^{2+}) into nanoparticles and as capping agents, stabilizing the formed nanoparticles to prevent aggregation

(Prasad and Elumalai, 2011). Within a few hours of mixing, a visible color change from light yellow to pale brown was observed, signalling the successful formation of ZnNPs due to the reduction of zinc ions by the phytochemicals (Jha *et al.*, 2009). The use of dry leaf material, as opposed to fresh leaves, demonstrated retained phytochemical activity, making this method viable for large-scale or off-season production, enhancing its practical applicability (Ahmed *et al.*, 2016). This approach aligns with green chemistry principles by eliminating the need for toxic chemical agents, thus reducing environmental impact and improving biocompatibility for potential applications.

To confirm the synthesis and characterize the ZnNPs, multiple analytical techniques were employed to assess their formation, size, structure, stability and surface properties. UV-Visible spectroscopy was used to analyze the nanoparticle suspension in the 200–800 nm wavelength range, revealing a sharp absorption peak at approximately 380 nm, characteristic of the SPR of ZnNPs (fig. 2). This SPR peak, resulting from the collective oscillation of conduction band electrons, confirmed the nanoscale nature of the particles and their relative stability, as the peak's intensity and sharpness indicated minimal aggregation (Singh *et al.*, 2020). The 380 nm peak falls within the typical 360–400 nm range for biosynthesized ZnNPs, with its position suggesting small, monodispersed particles, as variations in peak position (blue or red shifts) correlate with changes in particle size and aggregation state. No comparable absorption was observed in the control (plant extract alone), reinforcing that the peak was specific to ZnNP formation.

FTIR spectroscopy was conducted in the 4000–450 cm^{-1} range to identify the functional groups on the ZnNP surface, providing insights into the biomolecules responsible for reduction and stabilization (fig. 3). The FTIR spectrum displayed key absorption peaks: a broad peak at 3251.97 cm^{-1} (O–H stretching, indicating alcohols or phenols from polyphenolic compounds), 2920.5

cm^{-1} (C-H stretching of alkanes, likely from hydrocarbons in proteins or carbohydrates), 1602.86 cm^{-1} (N-H bending or C=C stretching, suggesting amide groups or aromatic rings from proteins or secondary metabolites), 1395.32 cm^{-1} (symmetric COO^- stretching, indicating carboxylic acids or amino acid residues) and 1045.66 cm^{-1} (C-O stretching, associated with alcohols, ethers, or esters from polysaccharides or other plant compounds). These peaks confirmed that phytochemicals, particularly polyphenols, proteins and carboxylic compounds, played a critical role in reducing zinc ions and capping the nanoparticles, enhancing their stability and bioactivity. The absence of prominent metal-oxygen peaks ($500\text{--}600 \text{ cm}^{-1}$) was attributed to the dominance of organic moieties or lower intensity of metal oxide vibrations.

SEM at 50,000x magnification was used to examine the surface morphology and size of the ZnNPs (fig. 4). The SEM micrographs revealed predominantly irregular, semi-spherical nanoparticles with a tendency to aggregate, forming clusters due to the high surface energy of the particles and the presence of bio-organic capping agents from the fenugreek extract (Sangeetha *et al.*, 2011). The estimated particle size was below 200 nm, as indicated by the scale bar, confirming their nanoscale dimensions. The observed agglomeration, a common feature in

green-synthesized nanoparticles, is attributed to the interaction of phytochemical capping agents with the nanoparticle surfaces, which, while promoting stability, can lead to clustering. Despite the irregular morphology and aggregation, the nanoparticles exhibited characteristics suitable for applications such as antimicrobial activity and environmental remediation, as their bio-organic coating enhances biocompatibility and functional reactivity compared to chemically synthesized nanoparticles (Alavi and Nokhodchi, 2022). The heterogeneous morphology is typical of green synthesis, contrasting with the uniformity of chemical methods but offering ecological and safety advantages for biological and environmental applications.



Fig. 1 : Biosynthesized nanoparticles: Showing color change in test and control conditions.

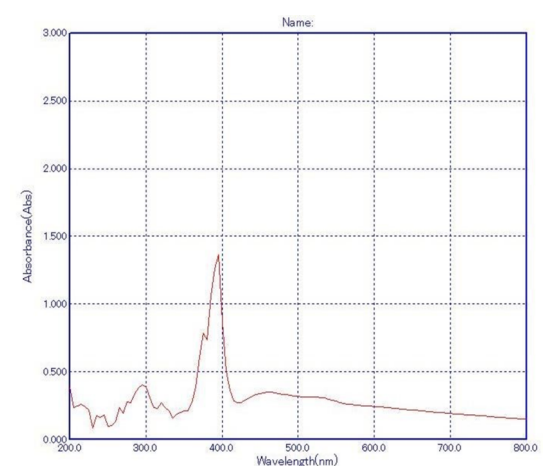


Fig. 2 : Spectral report of UV-VIS analysis of zinc nanoparticles.

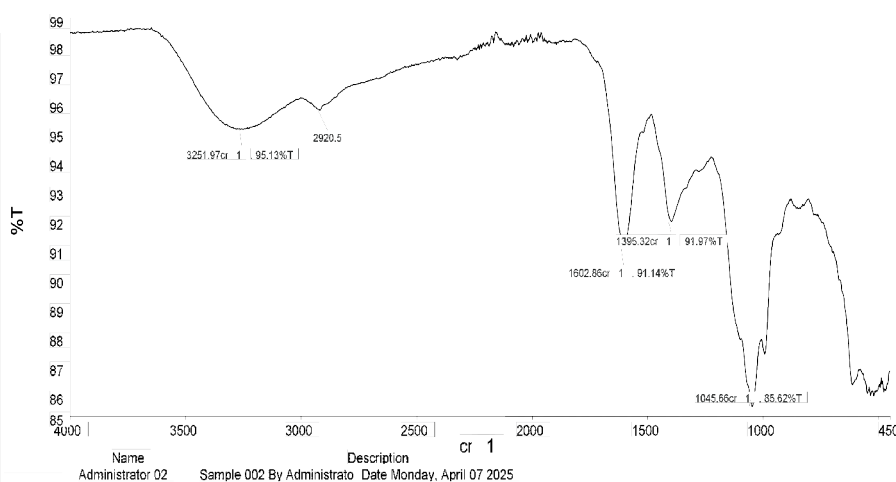


Fig. 3 : FTIR analysis of zinc nanoparticles

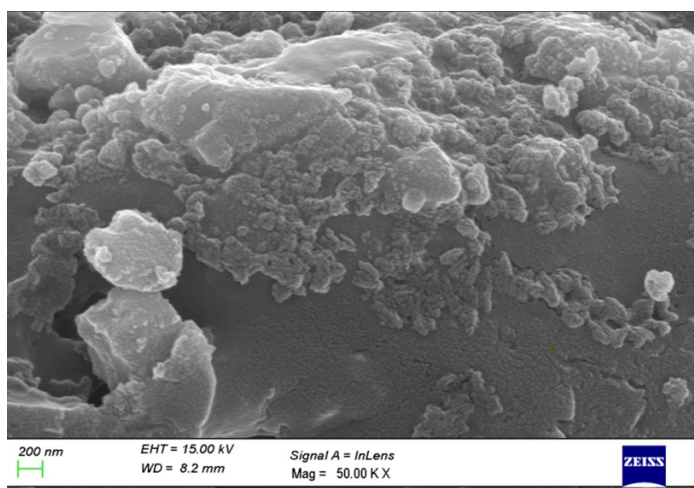


Fig. 4 : SEM image of Zinc nanoparticles showing structures at 50 K X magnification,

The AAS analysis (Fig. 5) confirmed the presence of heavy metals in soil samples. Lead (Pb) concentrations in plants varied significantly across treatments ($p < 0.05$), with the highest levels in H.M. (8.58 mg/kg), followed by N.P.

(8.36 mg/kg), C.F. (5.65 mg/kg) and the lowest in N.M. (2.16 mg/kg), indicating background Pb levels in uncontaminated soil. Multiple comparisons showed significant differences between N.M. and all other treatments, while H.M. and N.P. were statistically similar, suggesting comparable Pb uptake due to enhanced metal mobility and bioavailability, possibly influenced by nanoparticles. C.F. had a moderate effect on Pb uptake. ANOVA revealed significant differences in copper (Cu) accumulation ($p < 0.05$), with the highest levels in N.P. (8.47 mg/kg), followed by H.M. (8.32 mg/kg), C.F. (5.55 mg/kg) and N.M. (2.89 mg/kg) (Fig. 5). The increased Cu uptake in H.M. and N.P. treatments is likely due to higher availability and possible nanoparticle-induced chelation (Rajput *et al.*, 2018), while C.F. showed intermediate uptake due to fertilizer effects on soil properties.

Average heavy metal concentration

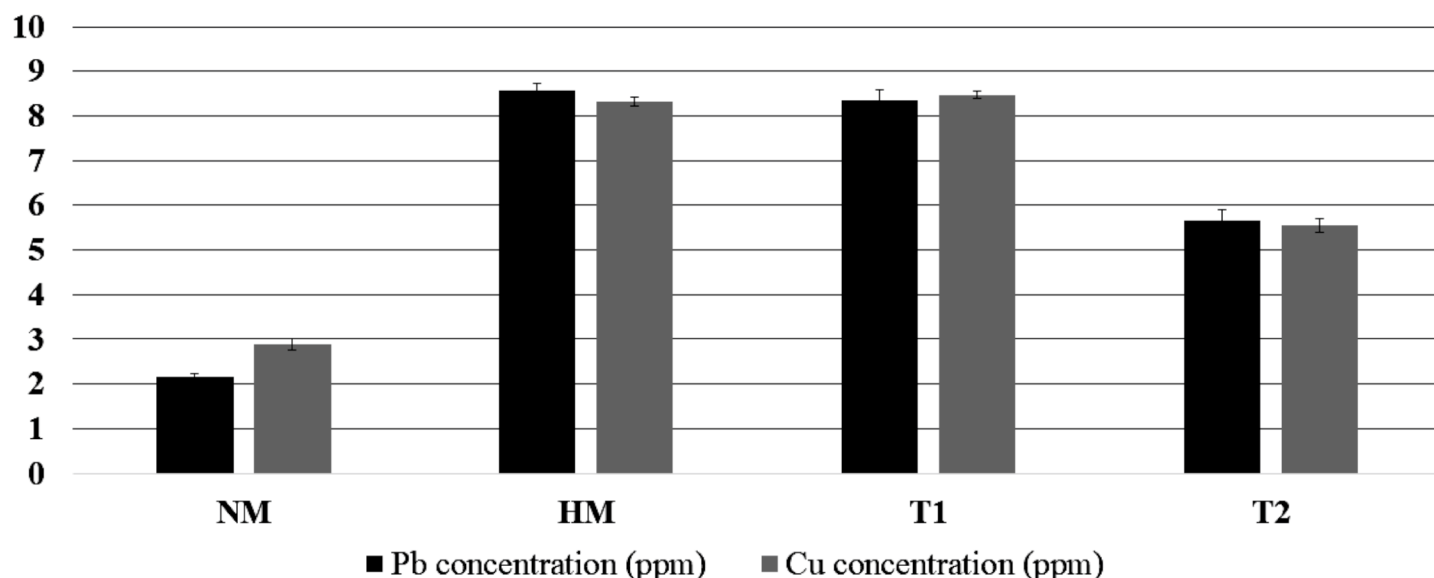


Fig. 5 : Concentration of heavy metals in control and treated plants.

The study evaluated the effects of control (NM) and treatments (HM, T1 and T2) on *Brassica juncea* growth, physiology and heavy metal uptake. Germination percentage was significantly higher in T1 and T2 compared to NM and HM, with T1 being the most effective, likely due to bio-stimulants mitigating heavy metal stress ($p < 0.05$). No significant differences were observed in

days to 50% flowering ($p = 0.177$), though HM delayed flowering slightly, consistent with stress-induced hormonal disruptions. Plant height was significantly increased by T1 (71.00 cm) compared to NM (59.07 cm), HM (55.33 cm) and T2 (55.47 cm) ($p = 0.004$), indicating T1's growth-promoting effects. Leaf length showed no significant differences across treatments ($p = 0.651$), despite

slight numerical variations. T1 significantly increased leaf number (19.67) compared to NM (10.67), HM (9.93) and T2 (13.60) ($p = 0.000$), reflecting enhanced vegetative growth. Leaf width was significantly higher in HM (3.93 cm) than NM (3.08 cm) ($p = 0.025$), possibly due to stress-induced morphological changes, but T1 and T2 showed no significant effects ($p = 0.148$). T1 significantly increased siliqua production (75.8) compared to NM (50.2), HM (57.2) and T2 (51.0) ($p=0.000$), suggesting improved reproductive efficiency. Seed count per siliqua was highest in T1 (9.73), significantly surpassing T2 (7.33) ($p = 0.024$), but overall treatment effects were not significant ($p=0.108$). Chlorophyll

content was highest in T1 (52.0–69.0), followed by T2 (42.0–58.0), with HM (27.0–40.0) showing a decline due to heavy metal toxicity. Lead (Pb) and copper (Cu) uptake was significantly higher in HM (8.58 mg/kg Pb, 8.32 mg/kg Cu) and T1 (8.36 mg/kg Pb, 8.47 mg/kg Cu) compared to NM (2.16 mg/kg Pb, 2.89 mg/kg Cu) and T2 (5.65 mg/kg Pb, 5.55 mg/kg Cu) ($p<0.05$), indicating enhanced metal bioavailability with nanoparticles. These findings highlight T1's potential in improving plant growth and phytoremediation under heavy metal stress, though further studies are needed to elucidate mechanisms and assess environmental risks.

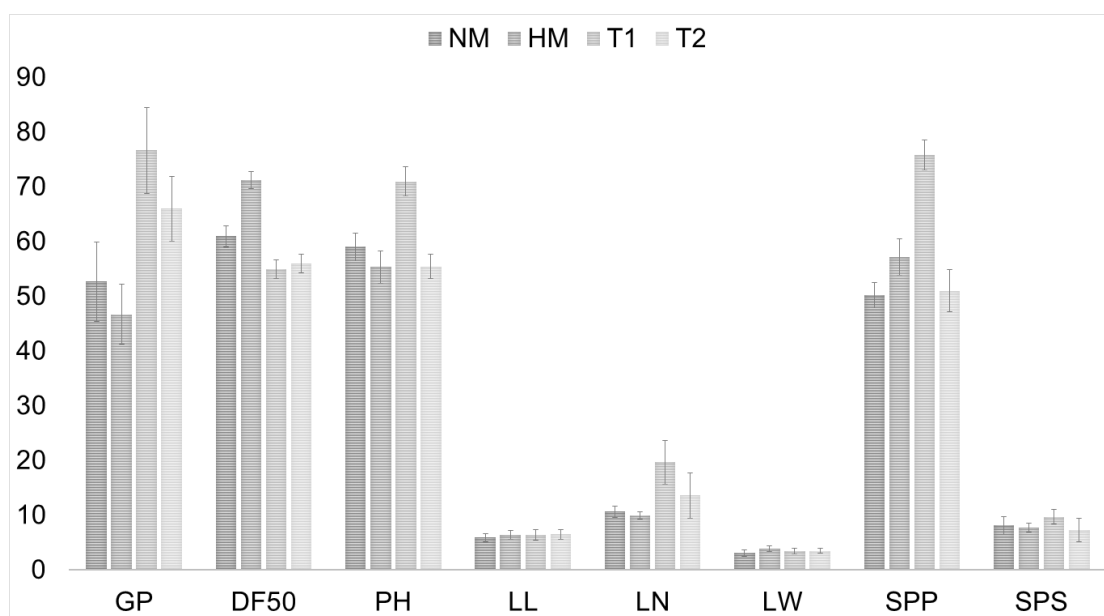


Fig. 6 : Average growth and yield parameters observed in control and treated plants.

GP= Germination percentage; DF50= Days to 50% flowering; PH= Plant height, LL=Leaf length; LN= Leaf number; LW= Leaf width; SPP= Siliqua per plant; SPS= seeds per siliqua.

Conclusions

The study demonstrated the efficacy of green-synthesized zinc nanoparticles (ZnNPs) using fenugreek leaf extract in enhancing the phytoremediation potential of *Brassica juncea* in soils contaminated with lead (Pb) and copper (Cu). Characterization via UV-Visible spectroscopy, FTIR, SEM and AFM confirmed the stability and favorable morphology of ZnNPs,

making them suitable for plant applications. In pot experiments comparing normal soil (N.M), heavy metal-contaminated soil (H.M.), ZnNP-treated contaminated soil (N.P.) and chemical fertilizer-treated contaminated soil (C.F.), ZnNP-treated plants exhibited significantly improved germination, earlier flowering, higher biomass and increased chlorophyll content compared to H.M. and C.F. treatments, indicating mitigation of heavy metal stress. Atomic absorption spectroscopy (AAS) revealed higher Pb and Cu accumulation in N.P.-treated plants, highlighting enhanced phytoextraction efficiency. ZnNPs

outperformed chemical fertilizers in both growth enhancement and metal uptake, likely by stimulating antioxidant enzyme activity and improving nutrient assimilation, thus reducing oxidative stress. This integrated approach of using green-synthesized ZnNPs with *B. juncea* offers a sustainable, eco-friendly and cost-effective strategy for remediating metal-contaminated soils, with potential to restore agricultural productivity in polluted regions.

Future research should validate these findings through large-scale field trials across diverse soil and environmental conditions to confirm practical applicability. Long-term studies are needed to assess the ecological fate of ZnNPs, including their interactions with soil microbes, potential groundwater leaching and food chain accumulation. Optimizing ZnNP dosage and formulation will be critical to maximize benefits while minimizing risks like phytotoxicity. Exploring other hyperaccumulator plants and biogenic nanoparticles could broaden the scope of nano-assisted phytoremediation for various contaminants. Molecular and genetic studies should investigate the mechanisms of ZnNP-induced stress alleviation and metal uptake to refine remediation strategies. Integrating ZnNP-assisted phytoremediation with sustainable practices like crop rotation and organic amendments could further enhance soil health and agricultural productivity in contaminated areas.

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Classical Genetics through a Mathematical Lens: Foundations, Genomic Relevance and Future Directions

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Abstract

Classical genetics, rooted in Mendelian principles and quantitative models, is a mathematically structured framework for studying heredity and phenotypic variation. From a mathematician's perspective, its elegance lies in transforming biological observations into algebraic and statistical systems. In the genomic era, classical genetics provides the scaffolding to interpret high-dimensional genomic data, bridging genotypes and phenotypes. This review defines classical genetics, highlights its enduring importance, and explores how mathematical tools like statistical genetics, algebraic geometry, persistent homology, and genetic algebras etc. advance its applications. These tools and their roles in modeling epistasis, recombination, and population dynamics has been summarized in this review. With the help of mathematical tools, classical genetics can address complex genetic interactions, enhancing future applications by emphasizing interdisciplinary mathematical approaches to unlock new insights into genetic systems.

Keywords: *Classical Genetics, Stochastic Processes, Algebraic topology, network theory, Bayesian Statistics and Birational Geometry*

Introduction

Genetics, originating with Gregor John Mendel's 19th-century experiments (1865) on pea plants, is the study of heredity based on observable inheritance patterns and phenotypic traits. Mendel's laws of segregation and independent assortment established the principles of how traits are passed from one generation to the next. However, the word genetic 'was coined in 1906, to designate the new science of heredity. Founded upon the Mendelian method for analyzing the products of crosses, this science is distinguished by its explicit purpose of being a general science of heredity. In the 1910s, Mendelian genetics fused with the chromosomal theory of inheritance, giving rise to what is still called 'classical genetics'. In the early 20th century, pioneers like T. H. Morgan, Ronald Fisher, Sewall Wright, and J.B.S.

Haldane expanded these concepts through linkage studies, biometrical models, and population genetics, formalizing the relationship between genes and phenotypes. This foundational connection established mathematics not just as a tool for data analysis, but as the very language for describing genetic principles.

Mathematics has always been integral to genetics, from Fisher's statistical models (1918) to Wright's path analysis (1921). In mathematician's view classical genetics is an achievement of formalizing biological phenomena into rigorous mathematical structures. Since from its origin, classical genetics models heredity through algebraic principles of segregation and assortment. Taking this mathematical interpretation forward, Ronald Fisher, Sewall Wright, and J.B.S. Haldane translate these ideas into statistical and algebraic frameworks, introducing concepts like heritability and linkage. These models transform the genotype-phenotype relationship into systems of equations, amenable to mathematical analysis.

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In the genomic era, where high-throughput sequencing generates vast datasets, classical genetics remains a cornerstone. Its mathematical foundations-rooted in probability, statistics, and algebra guide the interpretation of genomic data, from SNP associations to evolutionary dynamics. However, the complexity of epistasis, recombination, and high-dimensional data challenges traditional models, necessitating advanced mathematical tools.

Recent advances in mathematical frameworks, such as algebraic geometry and topological data analysis, offer new ways to model complex genetic interactions like epistasis and recombination. By exploring how these classical mathematical foundations have been extended and integrated with modern computational and statistical tools, such as machine learning and high-dimensional geometry, to address contemporary challenges in genomics and evolutionary biology, thereby demonstrating the enduring relevance of classical genetics in the era of genomics. This review defines classical genetics, underscores its relevance in genomics, and explores how mathematics can advance it.

Defining Classical Genetics

Classical genetics studies heredity through observable traits and inheritance patterns, formalized mathematically without molecular insights. Mendelian genetics, linkage and recombination, quantitative genetics and population genetics are considered as the core components of classical genetics. These components have been briefly reviewed below in context of the mathematical aspect

Mendelian Genetics: Mendel's experiments on pea plants established that traits are inherited as discrete units (genes) following two laws: segregation (alleles separate during gamete formation), and independent assortment (genes for different traits segregate independently). Crossing pea plants with purple (P) and white (p) flowers yields a 3:1 purple-to-white ratio in the F_2 generation. Mendel's laws (1865) are probabilistic models. Segregation implies alleles separate with equal probability (0.5) during gamete formation,

modeled as a binomial process (Teicher, 2014). Independent assortment treats loci as independent random variables, for a dihybrid cross (e.g., pea plant flower color and seed shape), the phenotypic ratio (9:3:3:1) follows a multinomial distribution.

Linkage and Recombination: Morgan's *Drosophila* studies (1910) showed that genes on the same chromosome are linked, inherited together unless separated by recombination. Recombination frequency, measured in centimorgans (cM), enables gene mapping. In linkage, genes on the same chromosome have dependent inheritance probabilities. Recombination frequency, θ , measured in centimorgans, defines a metric on the chromosome, enabling linear maps. The recombination probability is modeled as:

$$P(\text{recombination}) = \theta, 0 \leq \theta \leq 0.5$$

Quantitative Genetics: Fisher (1918), Wright (1921), and Haldane developed models for continuous traits (e.g., height, yield) influenced by multiple genes. Fisher (1918) formalized continuous traits using analysis of variance (ANOVA), partitioning phenotypic variance (V_P) into genetic (V_G) and environmental (V_E) components:

$$V_P = V_G + V_E + V_{G \times E}$$

Heritability, $h^2 = V_G / V_P$, quantifies genetic contribution. Wright's path analysis (1921) introduced causal graphs, modeling genetic effects as linear equations.

Population Genetics: The principles of Mendelian genetics, originally focused on individual crosses, were soon extended to populations. This shift required more sophisticated mathematical frameworks to model the dynamics of allele frequencies over time (Ewens, 2004).

The Hardy-Weinberg equilibrium models allele frequencies as a fixed point in a dynamical system, where p and q (allele frequencies) remain constant without selection or mutation. Selection dynamics are modeled using differential equations, e.g., for a single locus:



$$\Delta p = pqs / (1 + sq^2),$$

where s is the selection coefficient.

Foundational Mathematical Tools in Classical Genetics: A Mathematical Perspective

The study of heredity began with observations and folklore, but it was transformed into a predictive science by the rigorous application of mathematics. Mathematical tools have been indispensable for understanding and predicting genetic phenomena. The foundation of classical genetics is probability theory. Mendel's experiments revealed that the inheritance of traits followed predictable patterns, which could be expressed as simple ratios. The key mathematical and statistical methods that have shaped the study of classical genetics, highlighting their evolution from simple arithmetic and probability to complex algebraic structures and stochastic processes. Here we will discuss the foundational role of probability and combinatorics in Mendelian genetics, the application of algebraic and differential equations in population genetics, and the use of stochastic models to capture genetic drift and other random events.

Probability: The concept of a gene as a discrete unit of inheritance (now an allele) and the random segregation of these alleles during gamete formation are perfectly described by the rules of probability. For example, a cross between two heterozygous parents ($Aa \times Aa$) results in offspring with a genotypic ratio of 1:2:1 ($AA : Aa : aa$). This can be modeled using a Punnett square, which is a combinatorial tool, or by applying the product rule of probability:

- Probability of $AA = P(A) \times P(A) = 0.5 \times 0.5 = 0.25$
- Probability of $aa = P(a) \times P(a) = 0.5 \times 0.5 = 0.25$
- Probability of $Aa = P(A) \times P(a) + P(a) \times P(A) = 0.5 \times 0.5 + 0.5 \times 0.5 = 0.5$

Without probability, Mendel's law becomes a mere observation: "parents tend to pass on traits to their offspring." We lose the ability to predict the precise 3:1 phenotypic ratio in a monohybrid cross, or the 9:3:3:1 ratio in a dihybrid cross. The Punnett square, a simple but powerful combinatorial tool, would become a meaningless diagram without the associated probabilities.

Combinatorics: In a more complex scenario, such as a dihybrid cross, combinatorics becomes essential. The number of possible genotypes and phenotypes in the offspring can be calculated using principles of combinations and permutations. For example, a dihybrid cross ($AaBb \times AaBb$) yields 16 possible allele combinations, resulting in a 9:3:3:1 phenotypic ratio, a result that is a direct consequence of the laws of probability and combinatorics.

Algebraic Model: A fundamental concept in population genetics, the Hardy-Weinberg principle (1908), is an algebraic model that describes a non-evolving population. It states that in a large, randomly mating population with no mutation, migration, or selection, the allele and genotype frequencies will remain constant from generation to generation. The algebraic equation for a gene with two alleles, p and q , is:

$$p^2 + 2pq + q^2 = 1,$$

Here, p^2 represents the frequency of the homozygous dominant genotype, q^2 the frequency of the homozygous recessive genotype, and $2pq$ the frequency of the heterozygous genotype.

It provides a null hypothesis for evolution. Without this algebraic framework (Etherington, 1939), concepts like allele frequency, gene flow, and genetic drift become unquantifiable. How would one describe a population's genetic state without being able to measure the frequency of a given allele? The very idea of evolution as a change in allele frequency over time becomes a qualitative, observational statement rather than a testable, predictive theory.

Differential Equations: To model the forces of evolution that cause deviations from Hardy-Weinberg equilibrium, such as natural selection and mutation, geneticists turned to differential equations (Burger, 2000). For example, the change in allele frequency due to selection can be described by an equation that takes into account the fitness of different genotypes. This approach, pioneered by early population geneticists. R.A. Fisher and Sewall Wright, allowed for the quantitative analysis of evolutionary processes.



Stochastic Processes: While deterministic models based on differential equations are powerful, they fail to capture the random fluctuations in allele frequencies that occur in small populations. To address this, a new set of mathematical tools, known as stochastic processes, was introduced. The Wright-Fisher and Moran models are foundational stochastic models that describe genetic drift. These models use probability to simulate the random sampling of alleles from one generation to the next. The **Wright-Fisher model** (1931) assumes discrete, non-overlapping generations and describes the number of alleles of a certain type in the next generation based on a binomial distribution. On the other hand, the **Moran model** taken into the consideration of evolution of a population with a constant size and assumes overlapping generations and a continuous-time process, where an individual is chosen to reproduce and another to die in each time step (Moran, 1962).

These models, and the more generalized diffusion equations derived from them, have been instrumental in understanding how genetic variation is lost or fixed in populations due to chance alone (Fudenberg, 2004). In the real world, populations are not infinite, and evolution is not a perfectly deterministic process (Charlesworth and Charlesworth, 2010). Small populations are subject to genetic drift, the random fluctuation of allele frequencies due to chance. This phenomenon is incomprehensible without the tools of stochastic processes and probability theory (Kimura, 1955 a,b,c). Without models like the Wright-Fisher or Moran models, genetic drift would be relegated to an unexplainable "quirk" of small populations (Piegorsch, 1990). We would lose the ability to predict the probability of an allele becoming fixed or lost over time, making it impossible to model the dynamics of rare alleles or the loss of genetic diversity.

How Classical Genetics Can be Taken Forward with Mathematics in the Genomics Era

The genomic era, marked by technologies like next-generation sequencing and genome-wide association studies (GWAS), generates vast datasets of genetic variants (e.g., SNPs). Classical

genetics provides the framework to interpret these data by linking genotypes to phenotypes (Smýka *et al.*, 2016). The principles of classical genetics, when combined with advanced mathematical tools, can provide a more comprehensive and predictive framework for understanding genomic data. This integration allows us to move beyond simple correlations and build mechanistic models. This can be best understood with the given examples

Bridging Phenotype and Genotype with Algebraic Geometry: Genomics provides a "list" of all genetic variations (genotypes), but the challenge is to understand how these variations collectively produce a trait (phenotype). This is a complex, high-dimensional problem. Algebraic geometry, which studies solutions to systems of polynomial equations, offers a powerful framework to model how combinations of genetic variants interact to produce complex traits. This can help to formalize the concept of epistasis, a core challenge in both classical and modern genetics.

Algebraic geometry, which studies solutions to polynomial equations as geometric objects (varieties), offers a novel approach to model complex genetic interactions, particularly epistasis—where the effect of one gene depends on another. Epistasis is modeled as a polynomial system:

$$P = f(G_1, G_2, \dots, G_n)$$

Where, G_i are genotypes at loci, and P is the phenotype. The solution set (variety) maps genotype combinations to phenotypes.

Genetic algebras, introduced by Etherington (1939), model inheritance as algebraic structures where basis elements represent gametes and structure constants encode offspring probabilities. These align with algebraic geometry by representing genetic systems as polynomial systems, enabling analysis of complex inheritance patterns in populations. Algebraic geometry models these as a polynomial system, with persistent homology quantifying recombination events in natural populations, revealing why heterozygous variants persist. Tools like



Macaulay2 or R packages could make algebraic geometry accessible to geneticists.

Recombination pattern and Algebraic Topology: Persistent homology, a tool from algebraic topology, quantifies the “shape” of genetic data, such as recombination patterns. It generates barcodes summarizing topological features (e.g., loops representing recombination events). Persistent homology can extend classical linkage mapping to wild populations, informing conservation strategies. Chan *et al.* (2013) applied this to viral evolution, modeling influenza recombination to predict antigenic shifts, a technique adaptable to classical linkage studies. Persistent homology quantifies these events, identifying loci combinations that increase virulence, extending classical linkage mapping to genomic scales.

Integrating pedigree and SNP information with mixed linear models: Classical genetics relies heavily on statistics. Fisher's analysis of variance (ANOVA) partitions phenotypic variance into genetic and environmental components, enabling heritability estimation. Modern statistical tools, like mixed linear models used in GWAS, extend this framework to high-dimensional genomic data, integrating pedigree and SNP information.

Modeling Complex Inheritance with Network Theory: Many human traits and diseases are not determined by a single gene but by a complex network of interacting genes. Classical ideas like linkage and epistasis can be re-conceptualized in the language of network theory. Genes can be represented as nodes and their interactions as edges. Mathematical tools from network science can then be used to identify key regulatory genes, predict the effects of mutations, and understand how genetic networks respond to environmental changes. This moves beyond the linear thinking of classical genetics to a more holistic, systems-based view.

Predictive Modeling with Machine Learning: While classical genetics provided a simple predictive framework (e.g., Punnett squares), modern genomics demands more powerful predictive tools. Machine learning models, trained on large genomic and phenotypic

datasets, can learn complex relationships that are too subtle for human analysis. These models, however, are most effective when their architecture is informed by classical genetic principles. For instance, a model designed to predict disease risk might be structured to account for known genetic linkages or segregation patterns, thereby combining the power of modern computation with the fundamental rules of classical inheritance.

Bayesian Statistics and Birational Geometry: Bayesian models in genetics often face non-identifiability – multiple parameter sets yielding the same distribution. Birational geometry resolves singularities in these models, improving inference. Watanabe (2022) used this to refine GWAS models for type 2 diabetes, enhancing classical twin study estimates of heritability. Integrating classical pedigree analysis with genomic data and Bayesian models will improve disease risk prediction. Table 1 summarizes key mathematical tools and their applications in advancing classical genetics.

Conclusion

Classical genetics, rooted in Mendelian principles and quantitative models, remains vital in the genomic era, providing the conceptual framework to interpret complex traits and evolutionary processes. Its integration with mathematical tools like algebraic geometry, persistent homology, and Bayesian statistics enhances its ability to model epistasis, recombination, and population dynamics. By advancing classical genetics with mathematics, researchers can bridge genotype and phenotype, with machine learning one and model epistasis and predict complex traits more accurately. Similarly, persistent homology can extend classical linkage mapping to wild populations, informing conservation strategies. Despite its enduring relevance, classical genetics faces challenges in the genomic era. Combining pedigree-based classical methods with high-dimensional genomic data requires robust computational tools. Non-linear interactions like epistasis are difficult to capture with traditional statistics. Advanced mathematical tools like



algebraic geometry require interdisciplinary expertise. Continued interdisciplinary efforts will ensure classical genetics remains a cornerstone of biological discovery.

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From Nerves to Networks: Next-Generation Human-Machine Interfaces

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Abstract

Human-Machine Interfaces (HMIs) represent a transformative convergence of biology, neuroscience and engineering, enabling direct communication between the human body and external devices. This review explores HMIs from a biological perspective, emphasizing the fundamental role of neural, muscular and sensory systems in shaping interface design and function. It begins by outlining the neurophysiological mechanisms underlying electrophysiological signal processing, the musculoskeletal principles that support myoelectric control and the sensory pathways critical to creating responsive biofeedback loops. The review further delves into the importance of biomaterial biocompatibility, particularly in the context of implantable devices and long-term tissue integration. Current technologies – including brain-computer interfaces, wearable bioelectronics and neuroprosthetics – are examined for their therapeutic applications, particularly in restoring sensory or motor function in individuals with neurological or physical impairments. Emerging innovations such as AI-integrated systems, flexible bioelectronics and optogenetics offer a glimpse into future bio-integrated platforms that are more adaptive, personalized and less invasive. The paper concludes by highlighting key research directions, including synthetic biology, peripheral nerve modulation and ethical considerations surrounding neural augmentation. By placing biological principles at the core of interface development, this review underscores the importance of interdisciplinary collaboration in building HMIs that are not only functional but also harmoniously aligned with human physiology.

Keywords: Human-Machine Interface (HMI), Neurophysiology, Myoelectric Prosthetics, Biocompatible Materials, Brain-Computer Interface (BCI), Bioelectronics.

Introduction

The boundary between biology and technology is rapidly dissolving with the advent of Human-Machine Interfaces (HMIs), systems that allow direct communication between the human body and external machines. Once limited to the realm of science fiction, HMIs have evolved into a transformative field grounded in neuroscience, biomedical engineering and molecular biology. At their core, HMIs aim to interpret biological signals – especially neural, muscular, or bioelectrical – and translate them into actionable commands for prosthetic limbs, assistive devices,

or digital systems, enabling both therapeutic and augmentative applications (Lebedev and Nicolelis, 2017).

The biological foundation of HMIs lies in our intrinsic electrochemical nature. Human neurons transmit signals via action potentials, while muscles generate bioelectrical activity that can be externally recorded and interpreted. These physiological properties have enabled the development of technologies like brain-computer interfaces (BCIs), cochlear and retinal implants and myoelectric prosthetics that not only restore lost function but also increasingly mimic the natural sensation and responsiveness of biological systems (Schwartz *et al.*, 2006). In parallel, advances in materials science and tissue engineering have made it possible to design bio-integrative systems that can coexist within the

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body with minimal immune rejection, ensuring long-term compatibility and performance (Green *et al.*, 2019).

While the engineering challenges of HMIs are substantial, the biological questions they raise are equally compelling. How do neural circuits adapt to artificial inputs? What happens when synthetic devices become permanent parts of sensorimotor loops? How can we harness neuroplasticity to improve interface learning and control? These inquiries are not just technological—they are fundamentally biological, as they concern the adaptability, repairability and resilience of the human body.

Moreover, HMIs are not solely tools for rehabilitation; they increasingly promise enhancement. From cognitive augmentation to sensory expansion, these systems raise questions about the future of human identity, autonomy and the biological limits of the body (Yuste *et al.*, 2017). As HMIs evolve, so too must our understanding of the biological systems they interact with and the ethical frameworks governing their use.

This review aims to explore human-machine interfaces through a biological lens—examining their foundations in physiology, their current clinical applications and their future as biointegrated systems. Emphasis will be placed on neural, muscular and sensory pathways, as well as the interface materials and cellular responses that determine their success or failure.

Biological Foundations of Human-Machine Interfaces

The effectiveness of any Human-Machine Interface (HMI) hinges on its ability to interpret and respond to biological signals accurately and safely. At the heart of this interaction lie fundamental physiological systems—neural, muscular and sensory—whose complex, yet structured, bioelectrical communication networks provide the foundation for interfacing with machines.

Neurophysiology and Electrophysiological Interfacing

The nervous system operates through precisely

timed electrical impulses, or action potentials, transmitted by neurons. These impulses result from ion exchange across neuronal membranes, generating voltage changes that can be recorded and decoded (Kandel *et al.*, 2013). In HMIs, particularly in brain-computer interfaces (BCIs), these signals—captured via electroencephalography (EEG) or intracortical electrodes—serve as control inputs, allowing users to operate external devices directly from thought (Nicolelis and Lebedev, 2009). The fidelity of this neural interfacing is closely tied to our understanding of synaptic plasticity, neural coding and cortical mapping.

Musculoskeletal Biology in Myoelectric Interfaces

The musculoskeletal system also plays a pivotal role in HMIs, particularly through myoelectric prostheses. Muscle contractions are initiated by motor neurons releasing acetylcholine at the neuromuscular junction, triggering calcium-dependent interactions between actin and myosin filaments (Hall and Guyton, 2020). These contractions produce electrical signals—electromyograms (EMG)—that can be detected by surface or implantable electrodes and used to control prosthetic limbs. The specificity and strength of these signals determine the precision of limb movement and user comfort.

Sensory Biology and Biofeedback Integration

Equally crucial are the sensory systems, which enable biofeedback—a loop essential for intuitive control of machines. Mechanoreceptors in the skin, photoreceptors in the retina and proprioceptors in muscles provide continuous feedback to the brain about external stimuli and body positioning (Purves *et al.*, 2018). Integrating artificial sensors that mimic these receptors into HMIs helps create closed-loop systems that feel more natural, enabling users to sense pressure, temperature, or limb position in real time.

Biocompatibility and Implant Integration

Long-term success of implantable HMIs relies heavily on their biocompatibility. Materials must avoid eliciting chronic immune responses, inflammation, or fibrotic encapsulation



(Anderson *et al.*, 2008). Advances in biomaterials—such as conductive polymers, hydrogels and nanostructured surfaces—have improved tissue integration and signal fidelity, while reducing cytotoxicity and mechanical mismatch with soft tissues.

In sum, human-machine interfaces are not simply engineering marvels—they are deeply intertwined with the biology they aim to connect. Understanding these physiological principles is vital for designing systems that are intuitive, stable and safe for long-term use.

Neural Interfaces and Brain-Computer Interfaces (BCIs)

Among all categories of Human-Machine Interfaces (HMIs), Brain-Computer Interfaces (BCIs) stand out as the most direct and neurologically sophisticated form of communication between the brain and external devices. BCIs bypass conventional neuromuscular pathways by interpreting brain activity in real time, allowing users to control machines through thought alone. While initially conceptualized for medical rehabilitation, BCIs now represent a revolutionary platform for studying neural function, motor control and the brain's adaptability to technological feedback (Lebedev and Nicolelis, 2017).

Types of Neural Interfaces: Invasive vs. Non-Invasive

BCIs can be broadly categorized into non-invasive, minimally invasive and invasive systems, depending on the depth of neural signal acquisition. Non-invasive BCIs, such as electroencephalography (EEG), measure cortical activity through scalp electrodes. Although they offer safety and convenience, their spatial resolution is limited due to signal dispersion across the skull (He *et al.*, 2020).

Invasive BCIs, on the other hand, involve direct implantation of electrodes into the brain's cortex, such as with Utah arrays or microelectrode arrays (Schwartz *et al.*, 2006). These deliver high-resolution, single-neuron recordings essential for fine motor control, but raise issues of surgical risk,

biocompatibility and long-term neural tissue response (Fernández-León *et al.*, 2015). **Minimally invasive approaches**, like electrocorticography (ECoG), strike a balance by placing electrodes on the cortical surface, offering better fidelity than EEG with lower risk than fully invasive systems.

Signal Acquisition and Decoding

BCIs rely on the brain's electrophysiological signatures, typically from motor or premotor cortex, to decode user intent. Neural signals such as event-related potentials (ERPs), sensorimotor rhythms and spike trains are processed using machine learning algorithms that translate them into commands for external devices—ranging from computer cursors to robotic limbs (Wolpaw *et al.*, 2002). These interfaces often adapt over time as both the algorithm and the user co-train, highlighting the importance of **neuroplasticity** in optimizing BCI performance.

Clinical and Therapeutic Applications

One of the most profound uses of BCIs is in restoring communication and mobility to individuals with severe neuromuscular disorders such as amyotrophic lateral sclerosis (ALS), stroke, or spinal cord injury. BCIs have enabled “locked-in” patients to spell out words, navigate digital environments and control wheelchairs or exoskeletons (Birbaumer and Cohen, 2007). Moreover, BCIs are being used in **neurorehabilitation**, helping stroke survivors regain limb function through cortical re-engagement and neurofeedback (Ang *et al.*, 2015).

Ethical and Biological Challenges

Despite their promise, neural interfaces raise important ethical and biological concerns. Prolonged implantation can lead to gliosis, immune rejection, or signal degradation due to electrode encapsulation (Polikov *et al.*, 2005). Additionally, ethical questions regarding mental privacy, cognitive liberty and unintended modulation of mood or identity are increasingly being raised as interfaces grow more sophisticated (Yuste *et al.*, 2017).



BCIs offer more than control—they provide a biological window into the human mind, with implications extending from clinical neuroscience to cognitive augmentation. Their continued development will depend not only on engineering advances but also on deeper

Sensory Restoration and Augmentation Interfaces

One of the most impactful applications of Human-Machine Interfaces (HMIs) lies in the restoration and augmentation of sensory functions. Sensory HMIs are designed to replicate or enhance biological perception by interfacing directly with neural circuits or peripheral sensory systems. Whether enabling deaf individuals to hear, the blind to see, or amputees to feel, these systems blur the line between natural sensation and artificial input.

Cochlear Implants: Rewiring the Auditory System

Among the most successful sensory interfaces are cochlear implants, which bypass damaged hair cells in the cochlea by directly stimulating the auditory nerve using microelectrodes. These implants deconstruct incoming sound into frequency bands and convert them into electrical impulses that mimic natural hearing patterns (Wilson and Dorman, 2008). Over 700,000 individuals globally have benefitted from this technology, with children often achieving near-normal language development when implanted early (Sharma *et al.*, 2002). The biological integration of auditory pathways into a synthetic framework showcases how well-designed HMIs can effectively harness neural plasticity.

Retinal Prosthetics and Visual Interfaces

Retinal implants aim to restore vision in patients with degenerative retinal diseases like retinitis pigmentosa or age-related macular degeneration. These devices typically consist of photodiode arrays or electrode grids placed on the retina or optic nerve, converting light into electrical stimuli interpretable by the visual cortex (Luo and da Cruz, 2016). Although current resolution is limited, advances in microelectronics and neural

encoding strategies are steadily improving the quality of artificial vision.

Haptic Feedback and Tactile Perception

In prosthetic applications, haptic feedback systems have become essential for intuitive control. By embedding **mechanosensors** and delivering electrical or vibrational stimuli to residual limbs or sensory nerves, prosthetic users can "feel" textures, pressure, or temperature—restoring a sense of touch (Kim *et al.*, 2019). This closed-loop feedback dramatically improves motor control, reduces phantom limb pain and enhances user embodiment.

Augmenting Natural Sensation

Beyond restoration, HMIs are increasingly being explored for sensory augmentation—expanding human perception beyond its biological limits. Devices have been developed to translate infrared or ultrasonic signals into tactile or auditory cues, enabling users to perceive stimuli outside the natural sensory spectrum (Eagleman, 2015). These developments raise exciting prospects for merging biology with enhanced digital perception.

Sensory HMIs thus represent a profound confluence of neuroscience, engineering and biology, enabling not just recovery but also re-imagination of human sensation.

Bioelectronic Devices and Prosthetics

Bioelectronic prosthetics represent one of the most practical and life-transforming applications of Human-Machine Interfaces (HMIs). These devices integrate biological signals with electronic systems to **restore or enhance motor function**, particularly in individuals with limb loss or motor impairments. Unlike traditional prosthetics that operate passively, bioelectronic prostheses are dynamic, responsive systems capable of interpreting and executing user intent through neuromuscular or neural signals (Herr, 2014).

Myoelectric Prosthetics and Muscle Biology

Myoelectric prostheses function by detecting electromyographic (EMG) signals from residual



muscles. When a user intends to move a phantom limb, residual motor units still produce electrical activity that can be captured using surface or implanted electrodes (Farina *et al.*, 2014). These signals are decoded by onboard processors, which convert them into movement commands for motors controlling artificial joints. The specificity

and clarity of EMG signals are influenced by the anatomical distribution of motor units, skin impedance and training protocols—making muscle biology a critical determinant of prosthetic control quality.

Advanced myoelectric systems now employ pattern recognition algorithms to differentiate subtle

Table 1: Insights and applications of the various Biological system interfaces.

Technology	Biological System Interfaced	Signal Type	Signal Acquisition Method	Applications
Non-Invasive BCI (EEG)	Cerebral cortex (surface brain signals)	Electrical (EEG, ERPs, SMRs)	Scalp electrodes	Communication for ALS, basic device control
Minimally Invasive BCI (ECoG)	Cortical surface	Electrical (field potentials)	Subdural electrode grid	Motor prosthetic control, epilepsy monitoring
Invasive BCI (Utah Array, etc.)	Motor cortex (intracortical)	Electrical (spike trains)	Microelectrodes implanted into cortex	Robotic limb control, neurorehabilitation
Myoelectric Prosthetics	Residual skeletal muscles	Electromyographic (EMG)	Surface/implanted EMG electrodes	Upper/lower limb prostheses
Cochlear Implants	Auditory nerve	Electrical stimulation	Electrode array in cochlea	Hearing restoration in deaf individuals
Retinal Implants	Retina/optic nerve	Light → electrical signals	Photodiode or electrode array	Vision restoration for retinitis pigmentosa, AMD
Haptic Feedback Systems	Peripheral nerves, skin	Vibrotactile or electrical feedback	Mechanosensors + stimulators	Tactile sensing in prosthetics, VR
Biohybrid Interfaces	Cultured neurons or tissues	Electrical/biochemical	Living electrodes, bioelectrode arrays	Long-term implants, adaptive interfaces
Synthetic Biosensors	Engineered cells (e.g., bacterial, mammalian)	Electrical or optical	Gene circuits + nanoelectrodes	Smart implants, inflammation monitoring
Peripheral Nerve Interfaces	Peripheral nervous system	Electrical (nerve signals)	Cuff electrodes, regenerative interfaces	Organ function control, neuroprosthetics
Optogenetic Interfaces	Genetically modified neurons	Light-activated ion flux	Fiber optics + gene delivery	Precision stimulation for research, epilepsy treatment
AI-Integrated Interfaces	Brain, muscle, or hybrid systems	Multi-modal (neural, EMG, sensory)	ML-based signal decoding	Personalized neuroprosthetics, adaptive control
Flexible Bioelectronics	Skin, brain, or muscles	Electrical (biosignals)	Wearable or implantable thin films	Continuous monitoring, wearable HMIs

variations in muscle activity, allowing for more intuitive and multifunctional control. For instance, the LUKE arm, developed under DARPA's Revolutionizing Prosthetics program, can perform multiple grasp patterns with precise force modulation (Resnik *et al.*, 2018).

Biomimetic Design and Functionality

Modern bioelectronic prosthetics are increasingly **biomimetic**—designed to replicate the structure, movement and feedback mechanisms of biological limbs. Engineers use **soft robotics**, **tendon-like actuators** and **sensorized skin** to simulate natural movements and tactile perception (Kim *et al.*, 2013). These improvements not only enhance functional outcomes but also contribute to a stronger sense of embodiment and psychological acceptance among users.

Integration with AI and Neuroplasticity

Artificial intelligence (AI) and machine learning are central to improving prosthetic adaptation. AI enables continuous learning from user behavior, enhancing responsiveness and reducing cognitive load over time. Furthermore, studies show that long-term prosthetic use can induce **neuroplastic changes**, reinforcing the brain's capacity to incorporate artificial limbs into its motor and sensory maps (Makin *et al.*, 2013).

Bioelectronic prosthetics, therefore, exemplify the convergence of **biology**, **computation** and **mechanical design**. They serve not only as functional tools but also as test-beds for studying **neurophysiological adaptation**, human-machine synergy and embodied cognition in real-world scenarios.

Synthetic Biology and Living Interfaces

As Human-Machine Interfaces (HMIs) continue to evolve, a new frontier is emerging—the **integration of living systems with electronic devices**. This domain, grounded in synthetic biology and tissue engineering, seeks to create **living interfaces** where biological tissues do not merely tolerate machines, but actively participate in signal processing, adaptation and regeneration. These hybrid systems promise interfaces that are more **biocompatible**, **self-healing** and

functionally dynamic compared to conventional hardware-based designs (Green *et al.*, 2012).

Biohybrid Systems and Living Electrodes

Recent advancements have made it possible to develop **biohybrid devices**, where living cells are cultured on electrode platforms to enhance biological integration and signal fidelity. For instance, researchers are now embedding **neuronal cells on microelectrode arrays**, enabling real-time communication between biological networks and digital processors (Spira and Hai, 2013). These "living electrodes" not only reduce immune rejection but also allow devices to adapt with the host tissue through synaptic remodeling and neuroplastic responses.

Tissue-Engineered Interfaces

The use of **stem cells and biomaterials** to create **engineered tissues** that interface with prosthetics or neural implants is gaining momentum. For example, soft hydrogel scaffolds seeded with Schwann cells have shown promise in enhancing peripheral nerve regeneration and improving interface stability in long-term implants (Gu *et al.*, 2014). These constructs replicate the native microenvironment, allowing for **axon guidance**, **myelination** and **vascularization**—key elements for restoring sensory and motor continuity across the machine-body divide.

Electrogenic Cells and Cellular Biosensors

Synthetic biology also enables the design of **electrogenic cells** that can respond to stimuli such as light, temperature, or biochemical changes by generating electrical outputs. These genetically engineered cells can act as **biosensors**, converting physiological changes into digital signals (Rogers *et al.*, 2019). Such living sensors have potential applications in monitoring tissue health, metabolic changes, or inflammatory responses at the site of implantation.

Challenges and Opportunities

Despite the promise, living interfaces face **biological challenges** such as immune evasion, maintaining cell viability and ensuring reliable long-term function. However, the convergence of



Table 2: Advantages and limitations of the various Biological system interfaces.

Technology	Biological System Interfaces	Advantages	Limitations
Non-Invasive BCI (EEG)	Cerebral cortex	Safe, low-cost, portable	Low spatial resolution, signal noise, training required
Minimally Invasive BCI (ECoG)	Cortical surface	Better resolution than EEG, reduced risk compared to invasive	Still requires craniotomy, moderate immune response
Invasive BCI (Utah Array, etc.)	Motor cortex (intracortical)	High fidelity, single-neuron recording	Surgical risks, gliosis, limited long-term stability
Myoelectric Prosthetics	Residual skeletal muscles	Natural muscle signal use, non-brain-based control	Limited DOFs, requires training, affected by skin impedance
Cochlear Implants	Auditory nerve	High success rate, early implantation improves language outcomes	Limited frequency resolution, invasive surgery
Retinal Implants	Retina/optic nerve	Direct stimulation of visual system, expanding resolution tech	Visual quality still limited, surgical and power supply challenges
Haptic Feedback Systems	Peripheral nerves, skin	Improves embodiment, feedback for precision control	May require calibration, feedback may be unnatural initially
Biohybrid Interfaces	Cultured neurons or tissues	Biocompatible, self-healing potential, synaptic remodeling	Cell viability, immune control, difficult manufacturing
Synthetic Biosensors	Engineered cells (e.g., bacterial, mammalian)	High sensitivity, programmable, responsive to internal cues	Stability issues, risk of gene drift, ethical implications
Peripheral Nerve Interfaces	Peripheral nervous system	Less invasive than central interfaces, good specificity	Limited resolution, chronic implantation challenges
Optogenetic Interfaces	Genetically modified neurons	Cell-type specificity, high spatiotemporal control	Gene editing requirement, limited clinical use due to safety concerns
AI-Integrated Interfaces	Brain, muscle, or hybrid systems	Continuous learning, co-adaptive, improved accuracy over time	Requires large datasets, may overfit, black-box interpretability issues
Flexible Bioelectronics	Skin, brain, or muscles	Comfortable, conformable, minimally invasive	Power supply & durability in vivo remain concerns

synthetic biology, nanotechnology and regenerative medicine holds the potential to develop **self-regulating, adaptive HMIs** that redefine our relationship with machines—not as tools, but as extensions of our own living systems.

This synergy between engineered devices and biological tissue marks a shift from mechanical to **biointelligent interfaces**, opening new possibilities in rehabilitation, enhancement and real-time health monitoring.

Ethical, Social and Biological Considerations

As Human-Machine Interfaces (HMIs) advance from experimental technologies to clinically deployed tools—and even potential platforms for human enhancement—**ethical, social and biological considerations** become as critical as the technical innovations themselves. These interfaces do not merely extend function; they intersect with identity, autonomy and the fundamental nature of being human.

Biological Impacts and Long-Term Integration

From a biological standpoint, implantable HMIs must maintain long-term functionality without disrupting surrounding tissue. Chronic implants can provoke **immune reactions**, glial scarring and neuronal loss, especially in neural interfaces (Polikov *et al.*, 2005). While advances in soft and biocompatible materials reduce foreign body responses, the long-term physiological adaptation of the human body to these devices remains a concern. Additionally, the stability of signal acquisition may deteriorate over time, requiring recalibration or surgical intervention (Fernández-León *et al.*, 2015). Thus, designing devices that adapt to **neuroplastic changes**, rather than resist them, is a vital research priority.

Autonomy, Consent and Cognitive Integrity

HMIs capable of decoding brain activity or delivering sensory feedback raise profound **ethical questions** around **mental privacy** and **cognitive autonomy**. For example, closed-loop neurostimulation systems that treat depression or epilepsy may alter mood or personality in unintended ways (Fins *et al.*, 2017). This presents challenges for informed consent: how can patients

fully anticipate the cognitive and emotional consequences of a system that actively modifies their brain function?

Moreover, as interfaces become capable of **bidirectional communication**, the distinction between user and machine becomes increasingly blurred. Issues such as **agency, authorship of actions** and **responsibility** for behavior mediated by BCIs are topics of growing concern in neuroethics (Goering *et al.*, 2017).

Social Equity and Access

There is also the issue of **social justice**. Advanced HMIs, especially invasive ones, remain costly and are often only available in specialized centers. Without policies for **equitable access**, these technologies risk reinforcing healthcare disparities—benefiting a privileged few while marginalizing others. Furthermore, the commercialization of cognitive and physical augmentation raises questions about creating biologically enhanced "elites" in society.

Transhumanism and the Human Identity

Perhaps the most philosophical concern is the trajectory toward **transhumanism**—the use of HMIs not for restoration but for enhancement beyond biological norms. While this opens new dimensions of human capability, it also forces a re-evaluation of what constitutes **natural biological identity** and how we define “normal” human experience in a machine-integrated world (Yuste *et al.*, 2017).

Ethical stewardship, multidisciplinary regulation and inclusive public discourse must guide the future of human-machine integration—not merely to protect biology, but to ensure it evolves responsibly alongside technology.

Future Trends and Research Directions

The field of Human-Machine Interfaces (HMIs) is rapidly evolving, propelled by innovations in neuroscience, bioengineering and computational biology. As we move deeper into the 21st century, the trajectory of HMIs is likely to shift from assistive devices to integrated biological technologies that are seamlessly embedded within the human body and even the brain. This



section outlines key directions for future development, particularly emphasizing biologically relevant innovations.

Next-Generation Neural Interfaces

One major trend is the development of **minimally invasive or non-invasive brain interfaces** that retain high fidelity in signal acquisition. Traditional intracortical electrodes often suffer from long-term instability due to gliosis and tissue response (Polikov *et al.*, 2005). Emerging technologies such as flexible bioelectronic arrays, neural dust and optogenetics-based interfaces aim to interact with neurons more gently and precisely (Neely *et al.*, 2018). Optogenetics, in particular, offers cell-type-specific control using light, minimizing electrical noise and enhancing biological specificity (Boyden, 2015).

Synthetic Biology and Adaptive Feedback Systems

The integration of **synthetic biology** into HMI design opens new possibilities for real-time adaptation. Biohybrid systems using engineered cells may enable smart feedback loops that respond to changes in metabolism, inflammation, or cognitive state (Liu *et al.*, 2020). These systems can potentially self-regulate based on internal biological cues, vastly improving long-term compatibility and reducing device failure.

Peripheral Nervous System Interfaces and Regeneration

Another promising direction involves HMIs designed to work with the **peripheral nervous system (PNS)**. Recent research focuses on **bioelectronic medicines** that target specific neural circuits to modulate organ function—offering non-pharmacological treatments for chronic diseases like arthritis or hypertension (Tracey, 2014). Furthermore, regenerative approaches using stem cells, neural scaffolds, or electroceutical cues could help restore nerve-muscle communication in amputees or patients with neuromuscular disorders (Chen *et al.*, 2021).

Artificial Intelligence and Personalized Neurointerfaces

The incorporation of **artificial intelligence (AI)**

into HMI systems enables real-time pattern recognition and personalized calibration. Machine learning models can decode complex neural signals and adapt the interface to user-specific brain states, improving the efficiency and intuitiveness of device control (Abiri *et al.*, 2019). Future systems may become co-adaptive—learning from the user while facilitating neuroplastic changes in return.

Toward Bio-Integrated, Ethical Futures

Ultimately, the frontier of HMI research lies in **biologically integrated, ethically aligned** technologies that not only assist but empower individuals. As the line between biology and machine continues to blur, interdisciplinary collaboration—between neuroscientists, engineers, ethicists and clinicians—will be essential to ensure that these interfaces evolve to support human dignity and health.

Biological Harmony in Human-Machine Futures

Human-Machine Interfaces (HMIs) represent one of the most exciting interdisciplinary frontiers bridging biology, engineering and information science. From their earliest forms in rudimentary prosthetics to today's sophisticated brain-computer interfaces, HMIs have evolved to not only restore lost functions but also enhance and redefine human capabilities. At the core of this revolution lies an intimate understanding of biological systems—particularly the nervous, muscular and sensory networks—which enable seamless communication between living tissues and machines.

The biological foundations of HMIs—ranging from the electrophysiological dynamics of neurons to the contractile behaviour of muscles and the complex encoding of sensory inputs—are central to designing effective, responsive and user-centred devices. Technologies such as myoelectric prosthetics, brain-computer interfaces and biofeedback systems rely on precise modelling of these biological processes. Furthermore, advances in biomaterials have dramatically improved the compatibility of implants and wearables, minimizing immune

rejection and enhancing long-term stability.

Recent progress in bioelectronic devices and biocompatible sensors has further propelled HMIs toward clinical and consumer applications. Neural prosthetics, cochlear and retinal implants and biohybrid systems are now helping individuals overcome sensory or motor impairments with increasing efficiency and adaptability. The integration of artificial intelligence with biologically-informed feedback systems is paving the way for highly personalized and co-adaptive interfaces, capable of learning from and responding to individual users' unique neural signatures.

Looking forward, future trends suggest a shift toward minimally invasive, bio-integrated HMIs that leverage flexible electronics, synthetic biology and AI-based neural decoding. Such systems promise not only therapeutic benefits but also the possibility of cognitive augmentation, memory enhancement and expanded sensory perception. However, these advancements must be paralleled by ethical frameworks that address issues of privacy, consent and equitable access.

In essence, the success of HMIs depends not only on technological innovation but also on a deep respect for the biological systems they aim to interface with. As research continues to explore the boundaries of what it means to connect humans and machines, a biologically grounded, ethically guided approach will be essential in ensuring these technologies serve to enhance, rather than diminish, our shared humanity.

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Architecting Adsorbents: The Role of Texture–Chemistry Interplay in CO₂ Adsorption

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Abstract

The escalating concentration of atmospheric carbon dioxide (CO₂) necessitates the development of efficient capture technologies to mitigate climate change. Among various strategies, solid adsorbents have gained significant attention due to their tunability, reusability and lower energy demands compared to traditional amine-based solvents. However, the performance of these adsorbents is intricately governed by a combination of textural properties – such as surface area, pore size distribution and pore volume – and surface chemical features, including functional groups and binding affinities. This review comprehensively explores the synergistic interplay between these two domains in materials such as metal–organic frameworks (MOFs), covalent organic frameworks (COFs), activated carbons, mesoporous silicas and hybrid composites. Emphasis is placed on how tailored microporosity enables high capacity at low partial pressures, while functionalization with amines, nitrogen groups, or polar moieties enhances selectivity and adsorption strength. Strategies like post-synthetic modification, nitrogen doping and green synthesis from biomass are discussed. Key performance benchmarks – adsorption capacity, selectivity, kinetics, regeneration energy and stability – are evaluated using real-world data. The review also highlights recent advances in machine learning, in situ characterization techniques and sustainable material design. By integrating insights from both texture and chemistry, this work offers guidance for the rational design of next-generation CO₂ adsorbents with optimized performance for industrial deployment.

Keywords: CO₂ capture; solid adsorbents; adsorption selectivity; nitrogen doping; hybrid materials; machine learning; flue gas treatment.

Introduction

The alarming rise in atmospheric carbon dioxide (CO₂) concentrations – driven primarily by fossil fuel combustion – has positioned climate change as one of the most pressing challenges of the 21st century. Global CO₂ emissions reached approximately 36.8 gigatonnes in 2023, showing little sign of decline despite international mitigation efforts (Global Carbon Project, 2024). In response, carbon capture, utilization and

storage (CCUS) technologies have gained significant traction as viable tools for decarbonizing energy systems.

Among these, amine-based chemical absorption has been the most mature and widely implemented technique, particularly in post-combustion capture. However, this approach suffers from serious drawbacks, including high regeneration energy demand, corrosion and amine degradation under flue gas conditions (Boot-Handford *et al.*, 2014; Wang *et al.*, 2023). These limitations have spurred the development of **solid adsorbents** – materials that can physically or chemically bind CO₂ molecules – offering lower energy penalties and greater structural tunability.

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Solid adsorbents such as metal–organic frameworks (MOFs), zeolites, porous carbons, covalent organic frameworks (COFs) and functionalized silicas have emerged as promising alternatives due to their high surface areas, controllable pore structures and potential for chemical functionalization (Samanta *et al.*, 2012; Li *et al.*, 2021). However, adsorption efficiency depends not only on the material's textural properties—like surface area, pore volume and pore size distribution—but also critically on its surface chemistry, such as the presence of polar, basic, or amine-based functional groups (Sevilla and Mokaya, 2014; He *et al.*, 2019).

This review aims to explore the synergistic interplay between textural architecture and surface chemistry in solid adsorbents, emphasizing how their integrated optimization can enhance CO₂ uptake, selectivity and regeneration performance. Understanding this synergy is essential for guiding the rational design of next-generation carbon capture materials.

Fundamentals of CO₂ Adsorption

The mechanism of CO₂ adsorption on solid adsorbents can be broadly classified into two types: physisorption and chemisorption. *Physisorption* involves weak van der Waals interactions and typically occurs at low temperatures and high surface areas, enabling fast kinetics and easy regeneration. In contrast, *chemisorption* entails stronger covalent or ionic bonding—often involving surface functional groups such as amines—which provides higher selectivity and capacity but may require more energy for desorption (Goeppert *et al.*, 2012; Zhang *et al.*, 2020).

Role of Textural Properties

The textural architecture of an adsorbent—particularly surface area, pore size distribution and pore volume—plays a crucial role in governing CO₂ accessibility and diffusion. Micropores (<2 nm) are essential for capturing CO₂ due to size compatibility with CO₂ molecules (kinetic diameter $\approx$ 0.33 nm), while mesopores (2–50 nm) facilitate faster mass transport and reduce diffusion limitations (Li *et*

al., 2021). For example, activated carbons with hierarchical pores have shown superior performance due to the combination of high surface area (>1500 m²/g) and abundant micropores (Sevilla and Mokaya, 2014).

Role of Surface Chemistry

Beyond texture, surface chemistry directly influences adsorption affinity. Functional groups such as amines, hydroxyls, carboxyls, or nitrogen-containing moieties can form hydrogen bonds or acid–base interactions with CO₂ molecules, thereby enhancing chemisorption (Zhao *et al.*, 2013). Nitrogen-doped carbons, in particular, exhibit enhanced CO₂ binding through localized basic sites and electron-rich surfaces (Liu *et al.*, 2015).

Thermodynamics and Kinetics

Adsorption thermodynamics is typically expressed via the isosteric heat of adsorption (Q_{st}), which reflects the binding strength. Physisorption materials show Q_{st} values around 20–40 kJ/mol, while chemisorptive ones often exceed 60 kJ/mol (Choi *et al.*, 2011). Kinetics is influenced by pore accessibility and surface reactivity—faster diffusion in mesopores and prompt chemical interactions lead to higher performance.

Key Performance Parameters

Critical performance indicators for CO₂ adsorbents include adsorption capacity (mmol/g), selectivity (CO₂/N₂ or CO₂/CH₄), regeneration energy and working capacity (the usable uptake between adsorption and desorption conditions). Ideal adsorbents should exhibit high capacity at low partial pressures, rapid kinetics, high selectivity, low regeneration costs and robust cycling stability (Sumida *et al.*, 2012).

Textural Architecture: Its Role and Optimization

The textural architecture of an adsorbent—defined by its surface area, pore size distribution and pore volume—plays a pivotal role in determining the efficiency of CO₂ adsorption. Various porous materials such as activated



carbons, zeolites, metal-organic frameworks (MOFs), porous silica and covalent organic frameworks (COFs) have been developed with diverse textural characteristics to enhance gas adsorption performance (Sumida *et al.*, 2012; Li *et al.*, 2021).

Activated carbons, derived from biomass or synthetic precursors, are widely studied due to their tunable microstructure and cost-effectiveness. These materials often possess ultra-high surface areas ($>1500 \text{ m}^2/\text{g}$) and narrow micropores, which are particularly effective for low-pressure CO_2 adsorption because of the size compatibility with CO_2 molecules ($\sim 0.33 \text{ nm}$ diameter) (Sevilla and Mokaya, 2014). Similarly, zeolites—crystalline aluminosilicates—exhibit well-defined microporous frameworks that provide strong electrostatic fields conducive for CO_2 adsorption, especially at low partial pressures (Choi *et al.*, 2011).

However, reliance on micropores ($<2 \text{ nm}$) alone can lead to diffusion limitations. Incorporating mesopores ($2\text{--}50 \text{ nm}$) improves mass transfer and accelerates adsorption/desorption kinetics. For example, hierarchical porous MOFs like MIL-101 and UiO-66 derivatives offer dual porosity and high surface areas ($>3000 \text{ m}^2/\text{g}$), enabling both high capacity and rapid kinetics (Férey *et al.*, 2005; Yang *et al.*, 2017). Porous silica materials (e.g., SBA-15, MCM-41), while less selective, offer large mesopores and high chemical stability, making them suitable for functionalization and hybridization (Zhao *et al.*, 1998).

Importantly, surface area alone does not guarantee high CO_2 uptake. Studies show that pore size and volume are more critical than surface area when adsorption occurs under practical conditions (Nguyen *et al.*, 2013). A high proportion of narrow micropores ($0.4\text{--}0.7 \text{ nm}$) correlates strongly with uptake at flue gas conditions (0.15 bar CO_2), whereas mesopores improve accessibility and enhance working capacity under pressure swing adsorption (PSA) cycles (Wang *et al.*, 2020).

In summary, hierarchical porosity, which combines micropores for adsorption sites and

mesopores/macropores for enhanced diffusion, is essential for optimizing CO_2 capture performance. Rational design of tailored pore structures is key to balancing capacity, kinetics and regenerability in next-generation adsorbents.

Surface Chemistry: Functionalization Strategies and Mechanisms

The surface chemistry of an adsorbent plays a critical role in enhancing its interaction with CO_2 molecules, especially under low partial pressures and in selective capture scenarios. Incorporating polar and basic functional groups such as amines, hydroxyls, imidazole and pyridine significantly increases the binding affinity of adsorbents due to their ability to interact with the acidic nature of CO_2 through hydrogen bonding and acid-base interactions (Zhao *et al.*, 2013; Liu *et al.*, 2015).

Among these, amine functionalization is the most studied and effective approach. Primary and secondary amines react reversibly with CO_2 to form carbamates or bicarbonates, enhancing chemisorptive uptake and selectivity (Choi *et al.*, 2011). Materials like amine-grafted mesoporous silica (e.g., SBA-15-NH₂) and amine-appended MOFs (e.g., mmen-Mg(dobpdc)) have demonstrated high selectivity and isosteric heats of adsorption (Q_{st}) ranging from 60 to 90 kJ/mol, indicative of strong CO_2 binding (McDonald *et al.*, 2015).

Other polar moieties such as imidazole and pyridine groups in covalent organic frameworks (COFs) or nitrogen-doped porous carbons also exhibit enhanced CO_2 adsorption due to their Lewis basicity (Zhao *et al.*, 2017). Nitrogen doping introduces electron-rich sites that strengthen dipole-quadrupole interactions with CO_2 , improving uptake and selectivity without relying on strong chemisorption (Liu *et al.*, 2015).

Various surface modification techniques have been employed to incorporate these functional groups:

- **Amine grafting** on silica or MOFs using alkoxysilanes or post-synthetic ligand exchange.
- **Nitrogen doping** via thermal treatment of nitrogen-rich precursors (e.g., melamine, urea).



- **Oxidative treatments** to introduce hydroxyl and carboxyl groups, improving surface polarity and dispersibility (Sevilla and Mokaya, 2014).

These modifications significantly improve CO₂/N₂ and CO₂/CH₄ selectivity—critical for post-combustion capture. However, they often involve trade-offs. Chemisorptive sites, while highly selective, may reduce working capacity due to slow kinetics or high regeneration energy. For instance, strongly bound amines can lead to degradation over cycles and require elevated temperatures for desorption (Wang *et al.*, 2023).

Therefore, a balance between binding strength, reversibility and thermal stability is essential. Advanced designs now focus on hybrid systems that combine physisorption (for capacity) with chemisorption (for selectivity), aiming for optimal CO₂ capture performance.

Synergistic Design: Merging Texture and Chemistry

While high surface area has traditionally been associated with effective CO₂ capture, surface area alone is not a reliable predictor of performance. Without appropriate chemical functionalities, even materials with surface areas exceeding 3000 m²/g may demonstrate limited CO₂ adsorption due to weak physisorptive interactions (Nguyen *et al.*, 2013). This necessitates a synergistic design approach, integrating optimized textural features with surface chemistry to maximize uptake, selectivity and regenerability.

One of the most prominent examples of such synergy is found in amine-functionalized metal-

organic frameworks (MOFs). The prototypical mmen-Mg (dobpdc) combines high microporosity with pendant diamines, allowing for cooperative chemisorption of CO₂ with record capacities up to 3.9 mmol/g at 0.15 bar and 40 °C (McDonald *et al.*, 2015). Similarly, UiO-66-NH variants demonstrate enhanced CO₂ uptake due to their tunable porosity and amino-functionalized linkers (Lee *et al.*, 2013).

Nitrogen-doped porous carbons, particularly those derived from biomass (e.g., chitosan, urea, melamine), exhibit hierarchical porosity and built-in nitrogen sites. Liu *et al.* (2015) reported CO₂ uptake exceeding 4.6 mmol/g at 1 bar and 25 °C in N-doped carbon with micro- and mesoporous networks. These structures benefit from fast diffusion (enabled by mesopores) and high affinity binding (due to nitrogen doping), offering robust cycling and low regeneration energy.

Hybrid adsorbents, such as amine-impregnated mesoporous silica (SBA-15, MCM-41), have shown promising results by combining large pore volumes with tunable surface polarity. A study by Zhao *et al.* (2013) demonstrated 3.1 mmol/g CO₂ uptake at 1 atm for SBA-15 grafted with polyethylenimine. Similarly, polymer-carbon composites can integrate porous backbones with amine-rich polymeric chains to enhance capture.

To realize such synergy, several co-optimization strategies are used:

- **Post-synthetic modification:** For example, functionalizing MOFs or carbons with amines or ionic liquids.
- **Biomass-derived carbons:** Offer intrinsic

Table 1: Adsorbing materials and their CO₂ uptake.

Material	Surface Area (m ² /g)	CO Uptake (mmol/g)	Functional Group	Reference
mmen-Mg (dobpdc)	1200	3.9 @ 0.15 bar, 40°C	Diamine	McDonald <i>et al.</i> , 2015
N-doped porous carbon	1600	4.6 @ 1 bar, 25°C	Pyridinic N	Liu <i>et al.</i> , 2015
SBA-15 + PEI	~700	3.1 @ 1 atm, 25°C	Amine (PEI)	Zhao <i>et al.</i> , 2013
UiO-66-NH	~1000	2.2 @ 1 atm, 25°C	–NH	Lee <i>et al.</i> , 2013



heteroatoms (N, O, S) and hierarchical pores when carbonized under controlled conditions (Sevilla and Mokaya, 2014).

- **Computational tools:** Density functional theory (DFT) and machine learning models help predict binding affinities, pore-adsorbate compatibility and optimal functional group placement (Wilmer *et al.*, 2012).

This body of work underscores the necessity of integrated texture-chemistry engineering for developing scalable, high-performance CO₂ adsorbents.

Performance Benchmarks and Evaluation Criteria

To evaluate the viability of CO₂ adsorbents for practical applications, **multiple** performance criteria must be considered:

1. **CO₂ Adsorption Capacity:** Indicates the maximum amount of CO₂ adsorbed per unit mass, typically in mmol/g or wt%. Materials with >4 mmol/g at 1 bar are considered promising for post-combustion capture (Sumida *et al.*, 2012).
2. **Selectivity:** The ability to preferentially adsorb CO₂ over other gases like N₂ or CH₄. For example, amine-functionalized MOFs have CO₂/N₂ selectivity >100 due to strong acid-base interactions (McDonald *et al.*, 2015).
3. **Working Capacity:** The difference between adsorption and desorption under realistic pressure/temperature swing cycles. Hierarchical carbons and amine-grafted silicas have shown good working capacities with minimal hysteresis (Choi *et al.*, 2011).
4. **Regeneration Energy:** Low regeneration energy (<40 kJ/mol) is essential for economic viability. Physisorbents like N-doped carbons outperform strongly chemisorbing materials in this regard (Liu *et al.*, 2015).
5. **Thermal/Chemical Stability:** Materials must resist degradation under humid, thermal and acidic gas conditions over multiple cycles. For instance, UiO-66 derivatives retain >90% capacity after 50 cycles (Lee *et al.*, 2013).
6. **Scalability and Sustainability:** Biomass-

derived adsorbents and green-synthesized MOFs offer a lower environmental footprint and easier scale-up potential.

An “optimal” CO₂ adsorbent must meet a combination of structural, chemical and thermodynamic criteria to ensure efficiency, economic viability and scalability for real-world applications. The primary benchmark is high CO₂ uptake at low partial pressures, particularly around 0.15 bar CO₂, which reflects post-combustion flue gas conditions. For example, a threshold of ≥2 mmol/g at 0.15 bar and 25–40 °C is generally considered acceptable for industrial deployment (Samanta *et al.*, 2012).

1. **Selectivity** is another critical metric, especially CO₂/N₂ and CO₂/CH₄ ratios, which determine the material's performance in flue gas or natural gas streams. Amine-functionalized adsorbents and MOFs like mmen-Mg (dobpdc) can achieve CO₂/N₂ selectivities >100 (McDonald *et al.*, 2015).
2. **Kinetics**—both adsorption and desorption rates—must be fast to facilitate rapid cycling. This is influenced by pore accessibility and surface reactivity. Materials with hierarchical porosity (e.g., mesoporous carbons, MOFs) often exhibit faster diffusion (Nguyen *et al.*, 2013).
3. **Low regeneration energy** (<40 kJ/mol) is crucial for reducing operating costs. Physisorbents like nitrogen-doped porous carbons offer this advantage while maintaining acceptable uptake (Liu *et al.*, 2015). Lastly, thermal, mechanical and chemical stability, along with recyclability over ≥50 cycles, are essential for long-term use (Lee *et al.*, 2013).

A comprehensive evaluation must thus integrate adsorption capacity, kinetics, regenerability, selectivity and stability to identify candidates for industrial deployment.

Research gaps include improving performance under humid conditions, scaling up low-cost synthesis routes and balancing high selectivity with low regeneration penalties. Machine learning and high-throughput screening are



emerging tools to bridge these gaps and identify next-generation materials (Wilmer *et al.*, 2012).

Challenges and Future Directions

Despite significant progress in CO₂ adsorbent development, several critical challenges remain. Chief among them is the inherent trade-off between adsorption capacity, selectivity and regeneration energy. Materials that offer strong CO₂ binding (e.g., amine-functionalized MOFs or silica) often exhibit high isosteric heats of adsorption (>60 kJ/mol), resulting in greater regeneration costs and slower kinetics (McDonald *et al.*, 2015; Zhao *et al.*, 2013). Conversely, low-energy physisorbents may lack the selectivity required for flue gas separations.

Scalability and cost of synthesis methods is another bottleneck. High-performing materials like MOFs and COFs are often synthesized via multi-step, solvent-intensive processes that may be unsuitable for industrial-scale deployment. Addressing this requires development of green, solvent-free, or one-pot synthetic routes, particularly using biomass precursors or earth-abundant metal nodes (Sevilla and Mokaya, 2014).

Stability under realistic flue gas conditions—which often include water vapor, SO_x and NO_x—is a significant concern. Many MOFs and amine-based sorbents degrade or lose capacity over time when exposed to humidity or acidic gases (Sumida *et al.*, 2012).

Emerging solutions include machine learning-guided materials discovery, which enables rapid screening of structure-property relationships and predictive modeling (Wilmer *et al.*, 2012). Likewise, in situ spectroscopic tools such as IR, XPS and neutron scattering offer insights into adsorption mechanisms and material degradation in real-time.

Future research should also emphasize hybrid and composite materials, combining physisorptive backbones with chemisorptive functionalities to balance performance metrics. Finally, sustainability considerations, including life cycle assessments and renewable precursors, must guide next-generation adsorbent design for

global carbon capture efforts (Liu *et al.*, 2015).

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Natural Farming: A Pathway to Sustainable and Resilient Agriculture

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Abstract

Natural farming or zero budget natural farming is an eco-friendly agricultural approach that emphasizes minimal human intervention and the preservation of natural ecosystems to achieve sustainable food production. By avoiding synthetic fertilizers, pesticides and intensive tillage, natural farming fosters soil health, biodiversity and ecological balance. Techniques such as the use of organic amendments, cover cropping, mulching and indigenous microorganisms enhance soil fertility and water retention, reducing the carbon footprint of agricultural activities. This approach not only improves the biochemical quality and yield of crops but also contributes to long-term environmental sustainability and climate resilience. As a low-cost and resource-efficient model, natural farming offers a promising pathway toward carbon-negative farming and global food security, promoting the well-being of both farmers and ecosystems. The components involved in natural farming are beejamrit, jeevamrit, ghanjeevamrit, waaphas are used and for protection from disease and pest management Astra (Neemastra, Bhramhastra, Agniastra etc).

Keywords: Natural farming, Components, Disease and Pest Management

Introduction

Zero-Budget Natural Farming (ZBNF) was introduced in India in the 1990s by Shri Subhash Palekar (Korav *et al.*, 2020), who was honored with the Padma Shri award in 2016 for his contributions (Anonymous, 2016). ZBNF is a form of natural farming that eliminates the use of synthetic pesticides, fertilizers and other external inputs, promoting sustainable agricultural practices. The term "Zero Budget" reflects the approach of cultivating crops with minimal cost. By maintaining soil fertility and ensuring chemical-free production, ZBNF reduces production costs and enhances farmers' income.

ZBNF relies on organic preparations made from locally sourced materials like cow dung, cow urine and herbal solutions, complemented by practices such as crop diversification, mulching

and water-efficient techniques (Deshmukh and Akash, 2024). According to the Department of Agriculture & Farmers Welfare, 9.5 lakh hectares of land across 17 Indian states were under natural farming in 2022–2023, with around 20.08 lakh farmers engaged in these practices. Andhra Pradesh led with 2.9 lakh hectares, followed by Gujarat (1.86 lakh hectares) and Madhya Pradesh (1.11 lakh hectares) (Rajesh *et al.*, 2024).

Key components of ZBNF include Jeevamritha and Beejamritha, liquid fermented mixtures of cow dung, cow urine, jaggery, pulse flour and bund soil. Jeevamritha acts as a bio-stimulant, enhancing microbial activity in soil and on foliage, while Beejamritha is used for seed treatment to promote healthy plant growth. Other essential practices include the use of native seeds, intercropping and Achhadana (bio-mulching). Additionally, homemade biopesticides like Bramhastra, Agniastra and Neemastra are employed to manage pests such as fruit borers, stem and pod borers, leaf rollers, mealy bugs and sucking pests (Mishra, 2018). By fostering heterotrophic microbial communities and increasing soil organic matter, ZBNF significantly improves soil health (Shyam, *et al.*, 2019).

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Principles of natural farming

Natural farming is guided by core principles that emphasize working in harmony with nature to achieve sustainable agriculture. It avoids synthetic fertilizers, pesticides and herbicides, relying instead on organic inputs like cow dung, cow urine and plant-based solutions. Minimal soil disturbance through no-till practices preserves soil structure and enhances microbial activity, while mulching (Achhadana) conserves moisture, regulates temperature and suppresses weeds. Crop diversity through intercropping and rotation improves soil health and reduces pest outbreaks and water-efficient techniques like rainwater harvesting optimize resource use. Key bio-stimulants like Jeevamritha and Beejamritha promote beneficial microbial activity and healthy plant growth. By fostering biodiversity and maintaining ecological balance, natural farming enhances soil fertility, reduces production costs and supports long-term agricultural resilience.

Components of natural Farming

The key components of natural farming focus on enhancing soil health, promoting biodiversity and minimizing external inputs. **Jeevamritha** and **Beejamritha** are vital bio-stimulants made from cow dung, cow urine, jaggery, pulse flour and bund soil; Jeevamritha enriches the soil with beneficial microorganisms, while Beejamritha treats seeds for healthy germination. **Achhadana (mulching)** preserves soil moisture, prevents erosion and supports organic matter buildup. **Crop diversity** through intercropping and crop rotation strengthens ecosystem resilience and improves yield quality. **Water-efficient techniques**, like rainwater harvesting and micro-irrigation, ensure sustainable water use. Additionally, **homemade biopesticides** such as Bramhastra, Agniashtra and Neemastra help manage pests without chemicals. Together, these components create a self-sustaining agricultural system that maintains soil fertility, reduces costs and enhances crop productivity.

1. Beejamrit

Beejamrit is a crucial component of natural

farming, used for seed treatment to ensure healthy germination and protect young plants from soil-borne diseases and pests. It's a fermented organic preparation made from locally available ingredients like fresh cow dung, cow urine, lime, bund soil and water. This mixture is rich in beneficial microorganisms that coat the seeds, promoting strong root development and enhancing the plant's immunity. By encouraging microbial activity around the seed, Beejamrit helps the plants establish themselves quickly and grow more resiliently. As a low-cost, eco-friendly solution, it plays a vital role in sustainable agriculture, supporting chemical-free and high-quality crop production.

Beejamrit should be applied to any crop before the seeds, seedlings, or planting material are sowed or transplanted. After applying Beejamrit to the seeds, let them dry for a while. After the Beejamrit has dried on the seeds, the seeds should be sown. It works effectively to shield young roots from fungi, as well as from illnesses that are typically found in the nursery bed and are spread by seeds and dirt.



Fig. 1: Preparation of Beejamrit

Requirements :

1. Water - 20ltr
2. Desi Cow Dung - 5kg
3. Desi Cow Urine - 5Ltr
4. Lime - 50g
5. Fresh Soil from under the tree or forest - 500g

Methodology:

1. Take 20 litres water.

2. Then take 5 Kg Desi cow dung. Mix it by the fingers.
3. Take it in a cloth and bound it by small rope as a small bundle
4. Hang this bundle of cow dung in the taken 20-litre water for a night.
5. Take one litre water and add 50 gm lime in it, let it stable for a night.
6. Then next morning, squeeze this bundle of the cow dung in that water thrice continuously, so that all essence of cow dung will accumulate in that water.
7. Then add a handful of soil in that water solution and stir it well.
8. Then add 5 litre Desi cow urine or human urine in that solution
9. Then add the lime water and stir it well.
10. Keep it overnight for proper fermentation.
11. Now Beejamrita is ready to treat the seeds

2. Jeevamrit

Jeevamrit is a vital bio-stimulant in natural farming, designed to enrich soil health and promote microbial activity essential for plant growth. It's a fermented liquid mixture made from fresh cow dung, cow urine, jaggery, pulse flour, bund soil and water. This nutrient-rich preparation acts as a natural fertilizer, enhancing soil fertility by introducing beneficial microorganisms that help decompose organic matter and release essential nutrients. When applied to the soil or sprayed on plant foliage, Jeevamrit boosts plant immunity, improves nutrient absorption and supports the growth of healthy, chemical-free crops. As a cost-effective and eco-friendly input, it plays a key role in sustainable and carbon-negative farming systems.

It is a liquid manure that has fermented. Jeevamrit serves as a catalyst and provides nutrients to the soil. This causes the soils microbes to become more active. In addition, trees and plants can be shielded from bacterial and fungal infections with the use of jeevamrit. For one acre of land, 200 gallons of jeevamrit mixture are needed. Jeevamrit must be sprayed on the

farmers crops twice a month. It can be applied through drip irrigation or by combining it with irrigation water.



Fig. 2: Preparation of Jeevamrit

Requirements:

1. Water – 200 ltr
2. Fresh desi cow dung – 10 kg
3. Cow urine – 10 ltr
4. Jaggery – 2 kg
5. Flour of gram (tur, Moong, Cowpea) – 2 kg
6. Fresh soil from under the tree – 500 g

Methodology:

1. Take 200 litres water in a barrel for one acre crop utilization.
2. Add 10 kg cow dung in that water. Mix in desi cow dung in that water by the tips of your fingers well.
3. Stir it by a clock wise. Then add broken small pieces of jaggery.
4. Again, stir it well. Then add pulses flour in that solution.
5. Then add desi cow urine. Add handful soil under the tree in that solution.
6. Stir it well. Keep the cover of jute bag on the barrel.
7. Keep this solution quite stable for three days to ferment.
8. During fermentation, the poisonous gases like ammonia, methane, carbon-mono-oxide, carbon dioxide, are emitted. Through the holes of jute bag these gases are evacuated in

the atmosphere and aerobic fermentation process is going on with the high speed. For that purpose, we have utilized jute bag to cover the barrel.

9. Stir this solution by the branch of tree thrice a day.
10. Keep the barrel in shade or shadow. Do not expose jeevamrit to straight sunlight or rain.
11. Now jeevamrit is ready for utilization.

3. Ghanjeevamrit

Ghanjeevamrit is a solid, concentrated form of **Jeevamrit**, used in natural farming to enhance soil fertility and microbial activity over an extended period. It's prepared using cow dung, cow urine, jaggery, pulse flour and bund soil, similar to Jeevamrit, but without the large quantity of water. After mixing, the preparation is dried to create a compost-like material rich in beneficial microorganisms. Ghanjeevamrit slowly releases nutrients into the soil, improving its structure, increasing organic matter and promoting the growth of healthy, chemical-free crops.

Ghanjeevamrit can be stored for 6 month. Ghanjeevamrit is an organic fertilizer that can be prepared by mixing the following ingredients and allowing them to ferment:



Fig. 3: Preparation of Ghanjeevamrit

Requirements :

1. **Cow dung:** 100 kg
2. **Jaggery:** 1 kg
3. **Gram flour:** 2 kg
4. **Soil:** one handful
5. **Cow urine:** A bit of cow urine

Methodology:

1. Take 100 kg cow dung. Take 1 kg jaggery and make its powder.
2. Then mix it well in that cow dung.
3. Then take 2 kg flour of pulse and mix it in that cow dung properly.
4. Then mix handful soil under the tree. Then mix this mixture properly.
5. Add some cow urine in it if required. Keep it in the shadow for drying for 48 hours.
6. Cover it by gunny jute bag. Do not expose it to sunlight while drying. Dry it in the shadow.
7. After 48 hours let it to dry in the shadow. After drying crush it properly and then sieve it and store in the gunny bags.
8. Utilize this 200 kg ghanjeevamrit per acre either by spreading out before sowing or by sowing it with the seeds.

4. Waaphsa

Waaphsa is a key principle in natural farming that emphasizes maintaining the right balance of air and moisture in the soil to create an optimal environment for plant growth. Unlike conventional irrigation practices that often lead to waterlogging or overwatering, Waaphsa encourages minimal and efficient water use, ensuring that soil remains aerated and breathable. This balance supports the growth of beneficial microorganisms, enhances root development and prevents soil compaction. By reducing excessive water usage, Waaphsa also conserves resources and helps maintain soil structure, making it an essential practice for sustainable and resilient agricultural systems.

Aeration of the soil is necessary to preserve the necessary moisture-air balance, soil structure, appropriate irrigation and field water drainage.

5. Neemastra

Neemastra is an organic biopesticide used in natural farming to control a wide range of pests without harming the environment. It's made from neem leaves or neem pulp, cow urine, cow dung

and water – all easily available, natural ingredients. Rich in antifungal, antibacterial and insecticidal properties, Neemastra effectively repels pests like aphids, mealy bugs, caterpillars and sucking insects. When sprayed on crops, it not only protects plants from infestations but also boosts their immunity and promotes healthy growth. As a cost-effective, eco-friendly alternative to chemical pesticides, Neemastra plays a vital role in maintaining ecological balance and supporting sustainable, chemical-free agriculture.

Neemastra is used in crops to control aphids, jassids, mealy bugs, thrips, whiteflies, small caterpillars and other sucking pests. 250 liters of Neemastra is sufficient for spraying in one-hectare crop.



Fig. 4: Neemastra

Requirements:

1. Fresh Neem Leaves - 5kg
2. Desi Cow Urine -5Litre
3. Cow Dung -1kg
4. Water -100Litre

5. Brahmastra:

Brahmastra is very beneficial for protection from pests on crops. It is used to control large caterpillars and many other types of pests.

Requirements:

1. 10 litre cow Urine
2. 20 litre of water
3. 3 kg of Neem leaf

4. 2 kg of Karanj
5. 2 kg of Castor
6. 2 kg of Datura
7. 2kg of Calatropis



Fig. 5 : Preparation of Neemastra

Methodology:

1. To make Brahmastra, select any of the above five ingredients.
2. Grind all the leaves together well. Put this mixture in an earthen pot and boil it with 10 litres of water.
3. After 3-4 boil, take it down from the flame. Let it keep for 48 hours.
4. After this, mix 10litre of cow urine in it and filter it with a muslin cloth and keep it in an earthen pot to store.
5. It is important to protect it from the sun and the solution may be stored for up to 6 months.
6. Spraying should be done by mixing 2 to 3 litres of Brahmastra in 100 litres of water.

7. Agni Astra

Agni Astra is a powerful organic insecticide used in natural farming to manage a variety of pests without relying on harmful chemicals. It's prepared using cow urine, neem leaves, garlic, green chili and tobacco, creating a potent mixture with strong pesticidal properties. Agni Astra effectively repels and controls pests like caterpillars, aphids, mealy bugs and other sucking and chewing insects. When sprayed on crops, it not only eliminates pests but also boosts plant immunity and resilience. As a natural, low-

cost solution, Agni Astra supports sustainable farming practices by protecting crops while preserving soil health and ecological balance.

It is used to protect against the trunk borer, fruit borer and pod borer.



Fig. 6: Preparation of Agni Astra

Requirements:

1. 20 liters cow urine
2. 5 kg neem leaves
3. 500g tobacco powder
4. 500g green hot chilli
5. 500g garlic

Methodology:

1. Take a pot. Add 20 liter cow urine in it.
2. Crush 500 gm of green chilli, garlic, tobacco add it in cow urine.
3. Add 2 kg neem leaves.
4. Then boil this solution well 5 times. Let this solution to ferment for 24 hours.
5. Filter this by cloth.
6. Spray this agniastra on the pest like leaf roller, stem borer, fruit borer, pod borer.

Conclusion

Natural farming presents a sustainable and eco-friendly alternative to conventional agricultural practices, focusing on health, environmental harmony and long-term resilience. Despite certain challenges, its significant benefits for the

environment, economy and human well-being make it a compelling approach for the future of agriculture. By prioritizing organic methods, minimizing external inputs and maintaining ecological balance, natural farming has gained growing popularity among both farmers and consumers. Adopting its principles enables the development of productive, cost-effective and environmentally sound agricultural systems, fostering a healthier planet and a more sustainable food supply.

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Eco-friendly Nanofungicides: Biogenic Zinc Oxide Nanoparticles in the Management of Wheat Fungal Infections

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Abstract

This review explores the potential of biogenic zinc oxide nanoparticles (ZnO NPs) as eco-friendly nanofungicides for managing fungal infections in wheat, a crucial global crop. Traditional fungicides, while effective, pose environmental risks and contribute to pathogen resistance. Biogenic ZnO NPs, synthesized using green chemistry approaches, offer a sustainable alternative, combining the antifungal properties of zinc with the benefits of nanotechnology. The review discusses various biogenic synthesis methods, their impact on nanoparticle properties and the mechanisms through which ZnO NPs inhibit fungal growth. We also examine the current challenges and future prospects of deploying ZnO NPs in agricultural practices, emphasizing their potential to revolutionize wheat disease management.

Keywords: Nanofungicides, Zinc oxide nanoparticles, Green synthesis, *Triticum aestivum*, Sustainable agriculture.

Introduction

Wheat (*Triticum aestivum* L.) is one of the most critical cereal crops worldwide, serving as a staple food for billions of people and contributing significantly to global food security. As a major source of calories and nutrients, wheat underpins the diet of many populations and plays a pivotal role in the agricultural economies of numerous countries. However, wheat production faces substantial challenges, particularly from fungal diseases that can severely reduce yield and grain quality. Among the most notorious fungal pathogens affecting wheat are *Fusarium* spp., responsible for *Fusarium* head blight and *Puccinia* spp., which cause rust diseases such as leaf rust, stripe rust and stem rust. These pathogens not only diminish crop productivity but also pose health risks through the contamination of grains with mycotoxins, particularly in the case of *Fusarium* infections (Browne, 2007; McMullen, 2012).

Traditional methods for managing these fungal diseases predominantly rely on chemical fungicides, which have been instrumental in controlling pathogen spread and ensuring crop protection. However, the extensive and often indiscriminate use of these fungicides has led to several significant concerns. First, the development of resistant fungal strains has rendered some fungicides less effective over time, necessitating the application of higher doses or the development of new chemical formulations (Lucas, 2011). Second, chemical fungicides are associated with environmental contamination, affecting soil health, water quality and non-target organisms, including beneficial microbes and insects (Carvalho, 2017). These drawbacks highlight the urgent need for alternative disease management strategies that are both effective and environmentally sustainable.

In recent years, nanotechnology has emerged as a promising frontier in agricultural research, offering innovative solutions to enhance crop protection while mitigating the environmental impact of traditional practices. Nanoparticles, due to their small size and unique physicochemical

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properties, have shown potential in various agricultural applications, including as carriers for nutrients, pesticides and antimicrobial agents (Servin and White, 2016; Singh *et al.*, 2023). Among the different types of nanoparticles explored, zinc oxide nanoparticles (ZnO NPs) have garnered significant attention due to their inherent antimicrobial properties, biocompatibility and potential for large-scale synthesis (Rasmussen *et al.*, 2010; Kapoor *et al.*, 2018; Kapoor *et al.*, 2024). ZnO NPs exhibit a broad spectrum of activity against bacteria, viruses and fungi, making them suitable candidates for developing nanofungicides.

The synthesis of ZnO NPs can be achieved through various methods, including physical, chemical and biological approaches. Biogenic synthesis, which involves the use of biological entities such as plant extracts, microorganisms and enzymes, has gained popularity due to its eco-friendly nature and ability to produce nanoparticles with enhanced biocompatibility and controlled morphology (Goncalves *et al.*, 2021). Unlike chemical synthesis, which often involves toxic solvents and harsh reaction conditions, biogenic synthesis is a greener approach that leverages naturally occurring biomolecules to mediate the formation of nanoparticles. These biomolecules act as reducing, capping and stabilizing agents, influencing the size, shape and surface properties of the nanoparticles (Singh *et al.*, 2018).

Biogenic ZnO NPs have demonstrated potent antifungal activity against a variety of plant pathogens, including those affecting wheat. The antifungal mechanisms of ZnO NPs are multifaceted, involving the generation of reactive oxygen species (ROS), disruption of fungal cell membranes and the release of zinc ions, which can interfere with essential enzymatic processes within the fungal cells (Sirelkhatim *et al.*, 2015). Moreover, ZnO NPs have been shown to enhance the effectiveness of conventional fungicides, suggesting their potential role in integrated disease management strategies (Nair *et al.*, 2010).

This review aims to provide a comprehensive overview of the potential of biogenic ZnO NPs as

eco-friendly nanofungicides for managing fungal infections in wheat. We will explore the various biogenic synthesis methods, the mechanisms of antifungal action and the practical applications of ZnO NPs in field and laboratory settings. Additionally, we will discuss the challenges and future prospects of integrating ZnO NPs into sustainable agricultural practices. By examining these aspects, this review seeks to highlight the promise of ZnO NPs in revolutionizing wheat disease management and contributing to a more sustainable agricultural future.

Biogenic Synthesis of Zinc Oxide Nanoparticles

The biogenic synthesis of zinc oxide nanoparticles (ZnO NPs) represents a sustainable and eco-friendly alternative to conventional chemical and physical synthesis methods. This approach harnesses biological systems such as plant extracts, microorganisms and other biological agents to mediate the formation of ZnO NPs, offering several advantages, including reduced environmental impact, cost-effectiveness and enhanced biocompatibility of the resulting nanoparticles. The following sections provide an overview of the green synthesis approaches, the inherent advantages of biogenic synthesis and the characterization of the nanoparticles produced by these methods.

Green Synthesis Approaches

Biogenic synthesis of ZnO NPs is largely driven by the use of natural reducing agents present in plant extracts, microorganisms and other biological entities. These biological agents facilitate the reduction of zinc salts to ZnO NPs, acting as both reducing and stabilizing agents (Kapoor *et al.*, 2024). Among these, plant-mediated synthesis is particularly popular due to its simplicity, scalability and the wide availability of plant materials. Plant extracts contain a rich variety of phytochemicals, including flavonoids, alkaloids, phenols and terpenoids, which are responsible for the reduction of zinc ions (Zn^{2+}) to zinc oxide (ZnO) nanoparticles. For instance, extracts from *Aloe vera*, *Azadirachta indica* (neem) (Kapoor *et al.*, 2024) and *Moringa oleifera* have been



successfully used to synthesize ZnO NPs with desirable properties (Ramesh *et al.*, 2015; Kapoor *et al.*, 2017; Jayachandran *et al.*, 2020). The choice of plant species and extraction methods significantly influences the size, shape and surface properties of the nanoparticles, making this approach highly tunable.

Microbial synthesis is another promising green synthesis method, wherein bacteria, fungi and algae act as reducing and capping agents. Microorganisms secrete various enzymes and metabolites that facilitate the reduction of metal salts into nanoparticles. For example, *Bacillus subtilis* and *Fusarium oxysporum* have been employed to produce ZnO NPs with controlled morphology and enhanced antifungal properties (Kundu *et al.*, 2014; Sangeetha *et al.*, 2011). This method not only leverages the natural metabolic processes of microbes but also allows for the possibility of scaling up through fermentation techniques. Furthermore, microbial synthesis offers the advantage of genetic manipulation, enabling the optimization of nanoparticle production through the over expression of specific enzymes involved in the reduction process (Narayanan and Sakthivel, 2010).

Other biological agents, such as polysaccharides, proteins and even DNA, have been explored for the synthesis of ZnO NPs. These biopolymers provide functional groups that can bind zinc ions, facilitating their reduction and stabilization. For instance, the use of starch and chitosan has been reported to produce ZnO NPs with enhanced stability and uniformity (Singh *et al.*, 2018). These methods are particularly appealing for biomedical applications due to the biocompatibility and non-toxic nature of the starting materials.

Advantages of Biogenic Synthesis

The biogenic synthesis of ZnO NPs offers several key advantages over traditional chemical and physical methods. First and foremost, it is environmentally friendly, as it eliminates the need for toxic chemicals and harsh reaction conditions that are often employed in conventional synthesis processes. Traditional

methods typically involve the use of hazardous reducing agents like hydrazine and sodium borohydride, which pose significant environmental and safety risks (Raliya *et al.*, 2015). In contrast, biogenic synthesis utilizes natural reducing agents that are not only non-toxic but also biodegradable, minimizing the environmental footprint of the nanoparticle production process.

Cost-effectiveness is another major advantage of biogenic synthesis. The raw materials used, such as plant extracts and microbial cultures, are generally inexpensive and readily available. Moreover, the processes involved do not require sophisticated equipment or high energy inputs, further reducing the overall production costs (Kumar *et al.*, 2013). This makes biogenic synthesis an attractive option for large-scale production, particularly in resource-limited settings where access to advanced technologies may be restricted.

Enhanced biocompatibility is a critical advantage of ZnO NPs synthesized through biogenic routes. The biological agents used in the synthesis process often impart functional groups on the surface of the nanoparticles, which can improve their interaction with biological systems. For instance, nanoparticles capped with phytochemicals from plant extracts exhibit better biocompatibility and reduced cytotoxicity compared to those produced by chemical methods (Jiang *et al.*, 2018). This is particularly important for applications in medicine and agriculture, where the safety of the nanoparticles is paramount. Additionally, the surface functionalization provided by biological agents can enhance the stability and dispersion of ZnO NPs in various media, making them more effective in their intended applications (Rajiv *et al.*, 2013).

Characterization of Biogenic ZnO NPs

Characterizing the structural, morphological and chemical properties of biogenically synthesized ZnO NPs is crucial for understanding their behavior and efficacy in various applications. The synthesis method greatly influences these

properties, determining the size, shape, crystallinity and surface chemistry of the nanoparticles. Techniques including UV-Visible spectroscopy, electron microscopy (TEM and SEM), FTIR (Fourier Transform Infra-red spectroscopy), Dynamic light scattering (DLS), XRD (X-Ray diffraction analysis) etc. are done for the characterization of nanoparticles.

Morphologically, biogenic ZnO NPs can exhibit a wide range of shapes, including spheres, rods, hexagons and flowers, depending on the biological agent and synthesis conditions used. For example, plant-mediated synthesis often yields spherical or rod-shaped nanoparticles, while microbial synthesis can produce more complex structures like flower-shaped ZnO NPs (Bhuyan *et al.*, 2015, Kapoor *et al.*, 2018). These morphological differences can significantly impact the surface area and reactivity of the nanoparticles, influencing their antifungal activity and other functional properties.

Structurally, the crystallinity of ZnO NPs is a key determinant of their optical and electronic properties. X-ray diffraction (XRD) analysis is commonly used to assess the crystalline nature of biogenic ZnO NPs, which typically show a wurtzite crystal structure—a characteristic of ZnO. The degree of crystallinity can vary based on the synthesis method, with higher temperatures generally leading to more crystalline products (Jain *et al.*, 2016). The crystallite size, which can be estimated using the Debye-Scherrer equation, also varies with synthesis conditions and can range from a few nanometers to several tens of nanometers.

Chemically, the surface composition of biogenic ZnO NPs is influenced by the capping agents used during synthesis. Techniques such as Fourier-transform infrared spectroscopy (FTIR) and X-ray photoelectron spectroscopy (XPS) are employed to analyze the surface chemistry and identify functional groups present on the nanoparticle surface. These functional groups, derived from the biological agents used in synthesis, can provide stability and enhance the interaction of nanoparticles with biological targets, making

them more effective as antifungal agents (Kumar *et al.*, 2013).

In conclusion, biogenic synthesis methods offer a versatile and sustainable approach to producing ZnO NPs with desirable structural, morphological and chemical properties. By leveraging natural biological agents, these methods provide an eco-friendly alternative to traditional synthesis processes, resulting in nanoparticles that are not only cost-effective and biocompatible but also tailored for specific applications in agriculture, medicine and beyond.

Assessing the Antifungal Activity of ZnO NPs

The antifungal activity of ZnO nanoparticles (ZnO NPs) is commonly assessed through both *In vitro* and *in vivo* techniques, enabling evaluation of their effectiveness against plant pathogenic fungi. *In vitro* assays typically include the poisoned food technique, where ZnO NPs are incorporated into potato dextrose agar (PDA) medium and the inhibition of fungal mycelial growth is measured by comparing colony diameters of treated and untreated plates. Spore germination assays are also employed, wherein fungal spores are exposed to different concentrations of ZnO NPs and the percentage of germination inhibition is recorded microscopically. In broth dilution or poisoned broth methods, nanoparticles are added to liquid media to quantify their impact on growth of fungal biomass.

Microscopic observations are used to visualize morphological changes in fungal hyphae and spores after nanoparticle exposure. *In vivo* evaluation is conducted through seed priming, foliar spray, or soil application of ZnO NPs, followed by monitoring disease incidence and severity under greenhouse or field conditions. Biochemical markers and changes in antioxidant enzyme activities are also assessed to understand the mode of action. Collectively, these methods provide a comprehensive assessment of the antifungal efficacy of ZnO NPs, facilitating their comparison with conventional fungicides and establishing their potential as eco-friendly nanofungicides.



Mechanisms of Antifungal Activity of ZnO NPs

The antifungal efficacy of zinc oxide nanoparticles (ZnO NPs) has garnered significant attention due to their potent action against a wide range of fungal pathogens. The mechanisms through which ZnO NPs exert their antifungal effects are multifaceted, involving the generation of reactive oxygen species (ROS), disruption of fungal cell membranes, the release of zinc ions and synergistic interactions with other antifungal compounds. These mechanisms contribute to the inhibition of fungal growth and survival, making ZnO NPs a promising candidate for the development of nanofungicides.

Reactive Oxygen Species (ROS) Generation

One of the primary mechanisms by which ZnO NPs exert their antifungal activity is through the generation of reactive oxygen species (ROS). ZnO NPs can catalyze the formation of ROS, including hydroxyl radicals ($\cdot\text{OH}$), superoxide anions ($\text{O}_2^{\cdot-}$) and hydrogen peroxide (H_2O_2), under both UV light and ambient conditions. These ROS are highly reactive and can cause significant oxidative damage to fungal cells. The oxidative stress induced by ROS leads to the disruption of vital cellular processes, including DNA replication, protein synthesis and lipid peroxidation, ultimately resulting in cell death (Sirelkhatim *et al.*, 2015, Kapoor *et al.*, 2024).

The ROS generation by ZnO NPs is primarily attributed to their surface defects and the presence of oxygen vacancies, which facilitate electron transfer processes. When ZnO NPs come into contact with fungal cells, the ROS produced can penetrate the cell wall and interact with intracellular components, leading to oxidative stress. This stress disrupts the balance between the production and detoxification of ROS, overwhelming the fungal cell's antioxidant defense mechanisms and causing irreparable damage to cellular structures (Jin *et al.*, 2019). The high oxidative potential of ZnO NPs makes them particularly effective against fungi, which are generally more susceptible to oxidative stress compared to bacterial cells.

Cell Membrane Disruption

Another critical mechanism of antifungal action by ZnO NPs involves the direct interaction with fungal cell membranes. The cell membrane is the primary barrier protecting fungal cells from external threats and its integrity is essential for maintaining cellular homeostasis. ZnO NPs can interact with the lipid bilayer of fungal cell membranes, leading to structural disruptions that compromise membrane integrity. This interaction is facilitated by the small size and high surface area of ZnO NPs, which enable them to adsorb onto the membrane surface and penetrate the lipid bilayer (Nair *et al.*, 2010).

The disruption of the cell membrane by ZnO NPs can result in increased membrane permeability, leading to the leakage of essential intracellular contents such as ions, proteins and nucleic acids. This loss of cellular components disrupts the osmotic balance within the fungal cell, ultimately leading to cell lysis and death. Additionally, the interaction of ZnO NPs with the cell membrane can interfere with the function of membrane-bound enzymes and transport proteins, further compromising the cell's ability to maintain homeostasis (Padmavathy and Vijayaraghavan, 2008).

Ion Release and Nutrient Deprivation

The release of zinc ions (Zn^{2+}) from ZnO NPs plays a crucial role in their antifungal activity. Zn^{2+} ions are known to have antimicrobial properties and their release from ZnO NPs can significantly contribute to fungal inhibition. Once ZnO NPs are internalized by fungal cells or come into close contact with the cell membrane, the acidic environment within the cell can promote the dissolution of ZnO and the subsequent release of Zn^{2+} ions. These ions can disrupt various cellular processes by interfering with the function of metal-dependent enzymes, inhibiting protein synthesis and disrupting metabolic pathways (Sangeetha *et al.*, 2011).

Moreover, Zn^{2+} ions can lead to nutrient deprivation within fungal cells by binding to essential nutrients and cofactors, rendering them

unavailable for cellular functions. This nutrient deprivation can exacerbate the stress experienced by the fungal cell, further contributing to its death. The dual action of ZnO NPs, through both direct nanoparticle interaction and ion release, makes them highly effective in targeting fungal pathogens (Sirelkhatim *et al.*, 2015).

Synergistic Effects with Other Compounds

The antifungal efficacy of ZnO NPs can be further enhanced through synergistic interactions with other antimicrobial compounds, including conventional fungicides. Studies have shown that combining ZnO NPs with fungicides such as fluconazole or amphotericin B can result in a more pronounced antifungal effect than either agent alone. This synergistic action is likely due to the ability of ZnO NPs to disrupt fungal cell membranes, thereby facilitating the entry of fungicides into the cell. Additionally, the ROS generated by ZnO NPs can weaken the fungal cell, making it more susceptible to the action of fungicides (Jin *et al.*, 2019).

Synergistic effects have also been observed when ZnO NPs are used in combination with natural antimicrobial agents such as essential oils or plant extracts. These combinations can enhance the overall antifungal activity while potentially reducing the required dose of ZnO NPs and other agents, thereby minimizing potential toxicity and environmental impact (Raghupathi *et al.*, 2011). The ability to enhance the efficacy of existing fungicides through the use of ZnO NPs presents a promising strategy for overcoming fungal resistance and improving the sustainability of fungicide use in agriculture.

In summary, the antifungal activity of ZnO NPs is attributed to multiple mechanisms, including the generation of ROS, disruption of fungal cell membranes, release of zinc ions and synergistic interactions with other compounds. These mechanisms work in concert to inhibit fungal growth and ensure the effective management of fungal infections, making ZnO NPs a potent tool in the development of eco-friendly nanofungicides.

Application of Biogenic ZnO NPs in Wheat Disease Management

The application of biogenic zinc oxide nanoparticles (ZnO NPs) in the management of wheat diseases represents a promising strategy to combat various fungal pathogens that threaten global wheat production. Recent research has focused on assessing the efficacy of ZnO NPs against common wheat pathogens, such as *Fusarium* spp., which cause *Fusarium* head blight and *Puccinia* spp., responsible for rust diseases like leaf rust, stripe rust and stem rust. This section reviews the findings from field studies and laboratory trials, explores the modes of application for ZnO NPs in agriculture, analyzes their effectiveness in disease reduction and considers the environmental impact and safety of their use.

Field Studies and Laboratory Trials

Several laboratory and field studies have demonstrated the potential of ZnO NPs to manage fungal diseases in wheat effectively. Laboratory experiments have provided initial evidence of the antifungal properties of ZnO NPs against wheat pathogens (Wang *et al.*, 2016). ZnO NPs have been reported to reduce spore germination and mycelial growth by inducing oxidative stress through reactive oxygen species (ROS) generation (Rashid *et al.*, 2019).

Field trials have further validated the efficacy of ZnO NPs in controlling wheat diseases under natural conditions. In one study, ZnO NPs applied as a foliar spray significantly reduced the incidence of *Fusarium* head blight and rust diseases in wheat, leading to improved grain quality and yield (Elmer and White, 2018). The results indicated that ZnO NPs could effectively suppress disease development, particularly when applied at early stages of infection. Another field study found that wheat plants treated with ZnO NPs exhibited enhanced resistance to rust diseases, with lower disease severity and higher resistance compared to untreated controls (Khan *et al.*, 2020). These studies highlight the potential of ZnO NPs as a viable alternative to traditional fungicides in wheat disease management.



Mode of Application

The application of ZnO NPs in agriculture can be achieved through various methods, including foliar sprays, seed treatments and soil amendments. Each mode of application offers distinct advantages and can be tailored to specific agricultural practices and disease management strategies.

Foliar sprays are one of the most common methods for applying ZnO NPs to wheat crops. This approach involves spraying a suspension of ZnO NPs directly onto the leaves and stems of wheat plants, where the nanoparticles can act as a protective barrier against fungal pathogens. Foliar sprays are particularly effective in delivering ZnO NPs to the site of infection, allowing for rapid uptake and localized antifungal action. Studies have shown that foliar application of ZnO NPs can reduce the severity of wheat rust and *Fusarium* head blight by inhibiting spore germination and limiting fungal penetration into plant tissues (Elmer and White, 2018).

Seed treatments involve coating wheat seeds with a thin layer of ZnO NPs before planting. This method provides early protection against soil-borne pathogens, such as *Fusarium* spp., which can infect seedlings during germination. The ZnO NPs on the seed surface can inhibit fungal growth in the rhizosphere and prevent the establishment of infections that could lead to disease later in the plant's development (Rizwan *et al.*, 2019). Seed treatments with ZnO NPs have been shown to enhance seedling vigor, increase germination rates and improve overall plant health, contributing to better disease resistance throughout the growing season (Mousavi *et al.*, 2015).

Soil amendments involve incorporating ZnO NPs into the soil to provide long-term protection against fungal pathogens. This method allows for the gradual release of ZnO NPs into the soil, where they can interact with fungal spores and hyphae, inhibiting their growth and preventing the spread of infection. Soil amendments with ZnO NPs have been reported to reduce the

incidence of root and crown rot diseases in wheat by suppressing the activity of soil-borne pathogens (Ali *et al.*, 2019). Additionally, this approach can enhance nutrient availability in the soil, as ZnO NPs can release zinc ions, which are essential for plant growth and development.

Efficacy in Disease Reduction

The effectiveness of ZnO NPs in reducing disease incidence and severity in wheat has been demonstrated across various studies. ZnO NPs have shown a broad spectrum of antifungal activity, making them suitable for controlling multiple wheat pathogens simultaneously. For example, in a study on *Fusarium* head blight, the application of ZnO NPs reduced disease severity by up to 70%, significantly lowering the levels of mycotoxins in the harvested grain (Wang *et al.*, 2016). Similarly, in rust-infected wheat, ZnO NPs reduced the spread of the disease and decreased the overall infection rate, leading to healthier plants and higher yields (Rashid *et al.*, 2019).

The mode of application also influences the efficacy of ZnO NPs. Foliar sprays have been found to be particularly effective in managing above-ground fungal infections, such as rusts and blights, by providing direct protection to the plant's aerial parts. In contrast, seed treatments and soil amendments are more effective in preventing soil-borne diseases and ensuring early protection during seedling establishment (Mousavi *et al.*, 2015). The ability of ZnO NPs to reduce disease severity across different stages of wheat growth highlights their versatility as a disease management tool.

Environmental Impact and Safety

While ZnO NPs offer significant potential for disease management in wheat, their environmental impact and safety must be carefully considered. The widespread use of nanoparticles in agriculture raises concerns about their potential toxicity to non-target organisms, including beneficial soil microbes, insects and aquatic life. Studies have shown that high concentrations of ZnO NPs can have detrimental effects on soil microbial communities, potentially disrupting nutrient cycling and soil health (Raliya



et al., 2017). Therefore, it is crucial to optimize the concentration and application methods of ZnO NPs to minimize their environmental footprint.

Ecotoxicological studies have indicated that ZnO NPs can accumulate in the soil and water systems, posing risks to the broader ecosystem (Liu *et al.*, 2015). To mitigate these risks, researchers are exploring the use of biodegradable coatings for

ZnO NPs and developing slow-release formulations that reduce the likelihood of environmental contamination (Rizwan *et al.*, 2019). Additionally, the use of biogenic ZnO NPs, synthesized through environmentally friendly methods, can further enhance the safety and sustainability of their application in agriculture.

Table 1: Overview of Various Nanoparticles Used as Nanofungicides

Type of Nanoparticle	Size (nm)	Effective Against	Authors	Year
Silver Nanoparticles	10-30	<i>Fusarium oxysporum</i>	Jo, Kim, Jung and Kim	2009
Zinc Oxide Nanoparticles	20-60	<i>Candida albicans</i>	He, Zhang and Zeng	2011
Copper Nanoparticles	50-100	<i>Alternaria alternata</i>	Ahamed, AlSalhi and Siddiqui	2010
Titanium Dioxide Nanoparticles	25-40	<i>Botrytis cinerea</i>	Mousavi Kouhi, Lahouti, Ganjeali and Entezari	2015
Gold Nanoparticles	15-25	<i>Aspergillus niger</i>	Sharma, Yngard and Lin	2009
Silicon Dioxide Nanoparticles	12-50	<i>Penicillium expansum</i>	Kim, Kuk, Yu, Kim, Park and Hwang	2007
Magnesium Oxide Nanoparticles	30-80	<i>Fusarium graminearum</i>	Wang, Wei and Wang	2016
Copper Oxide Nanoparticles	10-80	<i>Sclerotinia sclerotiorum</i>	Villamizar-Gallardo, Mendoza-Mendoza and López	2018
Iron Oxide Nanoparticles	20-50	<i>Rhizoctonia solani</i>	Abdel-Aziz, Shaheen and El-Nekeety	2017

The application of biogenic ZnO NPs in wheat disease management offers a promising alternative to conventional fungicides, with demonstrated efficacy in laboratory and field settings. The versatility of ZnO NPs in different modes of application, coupled with their broad-spectrum antifungal activity, makes them a valuable tool for protecting wheat crops from various fungal pathogens. However, careful consideration of their environmental impact and safety is essential to ensure the sustainable use of ZnONPs in agriculture.

Challenges and Future Prospects

The application of biogenic zinc oxide nanoparticles (ZnO NPs) in wheat disease management holds significant promise for sustainable agriculture. However, several challenges must be addressed to realize the full potential of these nanofungicides, including scalability, cost-effectiveness, regulatory and safety concerns and integration into broader agricultural practices. Furthermore, future research needs to focus on understanding the long-term environmental impacts and the potential development of resistance by pathogens.



Scalability and Cost-effectiveness

One of the primary challenges in the widespread adoption of biogenic ZnO NPs is scalability. While laboratory-scale production of ZnO NPs using biological methods is relatively straightforward, scaling up these processes to meet agricultural demands poses significant technical and economic hurdles. The cost of producing ZnO NPs on a large scale can be prohibitively high due to the need for controlled synthesis conditions, purification processes and consistent quality control (Raliya *et al.*, 2017). Additionally, the sources of biological materials, such as plant extracts or microbial cultures, must be available in large quantities, which may not always be feasible. Developing more efficient, cost-effective methods for biogenic synthesis is crucial for making ZnO NPs a viable option for large-scale agricultural applications. Strategies such as optimizing the use of locally available and renewable biological resources, improving synthesis efficiency and reducing energy inputs can help lower production costs (Rizwan *et al.*, 2019).

Regulatory and Safety Concerns

The deployment of ZnO NPs in agriculture also raises important regulatory and safety concerns. Currently, there is a lack of comprehensive regulatory frameworks specifically addressing the use of nanomaterials in agriculture. This gap poses a significant challenge for the safe and responsible deployment of ZnO NPs, as the potential risks to human health, non-target organisms and the environment are not fully understood (Rizwan *et al.*, 2019). There is a pressing need for regulatory bodies to establish guidelines that cover the production, application and disposal of nanomaterials in agricultural settings. These regulations should be informed by rigorous safety assessments that consider the unique properties of nanoparticles, such as their size, shape and surface chemistry, which can influence their behavior in biological and environmental systems (Kah *et al.*, 2018). Moreover, standardized testing protocols must be developed to evaluate the ecotoxicological effects of ZnO NPs on soil health, water quality and biodiversity. Ensuring that ZnO NPs are safe for use in agriculture will require a collaborative

effort between researchers, industry stakeholders and regulatory agencies.

Integration into Sustainable Agriculture Practices

Integrating ZnO NPs into sustainable agriculture practices involves combining these nanofungicides with other eco-friendly approaches to create comprehensive, integrated disease management strategies. ZnO NPs have the potential to complement existing sustainable practices, such as organic farming, biological control and the use of resistant crop varieties. For example, ZnO NPs could be combined with biocontrol agents, such as beneficial fungi or bacteria, to enhance their antifungal efficacy while reducing the need for chemical fungicides (Khan *et al.*, 2020). Similarly, ZnO NPs could be used in conjunction with crop rotation and soil conservation practices to improve plant health and resistance to diseases. The integration of ZnO NPs into these systems requires careful consideration of the specific agricultural context, including crop type, environmental conditions and the presence of other management practices. Developing customized solutions that leverage the strengths of ZnO NPs while minimizing their environmental footprint will be key to their successful adoption in sustainable agriculture.

Conclusion

The review highlights the promising potential of biogenic zinc oxide nanoparticles (ZnO NPs) as an innovative and sustainable solution for managing fungal diseases in wheat. Through various studies, ZnO NPs have demonstrated potent antifungal activity against significant wheat pathogens like *Fusarium* spp. and *Puccinia* spp., primarily by generating reactive oxygen species (ROS), disrupting fungal cell membranes and releasing zinc ions that inhibit fungal growth. The ability to synthesize these nanoparticles using eco-friendly, biological methods further enhances their appeal, offering a safer alternative to traditional chemical fungicides that are associated with environmental pollution and the development of pathogen resistance (Sirelkhatim *et al.*, 2015; Khan *et al.*, 2020).

The implications for future agriculture are significant, as the integration of nanotechnology,

particularly biogenic ZnO NPs, could revolutionize crop protection strategies. By reducing reliance on conventional fungicides, which pose risks to both human health and the environment, ZnO NPs represent a critical step towards more sustainable agricultural practices. Furthermore, their potential compatibility with other eco-friendly approaches, such as organic farming and biological control, underscores the versatility of ZnO NPs in integrated disease management systems (Elmer and White, 2018).

However, to fully realize the benefits of ZnO NPs in agriculture, further research is essential. This includes exploring scalable production methods, conducting comprehensive safety assessments and investigating the long-term environmental impacts of these nanoparticles. Moreover, it is crucial to establish regulatory frameworks that ensure the safe use of nanomaterials in agricultural settings (Rizwan *et al.*, 2019). The adoption of green nanotechnologies, such as biogenic ZnO NPs, represents a forward-looking approach that aligns with global efforts to enhance food security while preserving environmental integrity. Researchers, policymakers and agricultural stakeholders must collaborate to promote and implement these innovations in a manner that maximizes benefits and minimizes risks.

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Exploring the Anti-Cancer and Antioxidant Properties of Jackfruit (*Artocarpus heterophyllus*) Seeds

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Abstract

This research delves into the pharmaceutical and health applications of extracts from *Artocarpus heterophyllus* (Jackfruit) seeds. Employing a comprehensive approach of molecular docking simulations and experimental assays, this study examines the binding potential, antioxidant activity, and cytotoxic effects of the extracts. The successful validation of the docking protocol ensures reliability. Notably, Kaempferol D exhibits high docking scores, indicating promise as a human tubulin inhibitor. Analysis of phenolic and flavonoid content reveals their abundance, particularly in the n-hexane extract. These compounds exhibit antioxidant properties, suggesting the extracts as natural antioxidant sources. Validation through the DPPH radical scavenging assay confirms antioxidant potential in both ethanol and n-hexane extracts. Furthermore, cytotoxicity assessment using the MTT assay highlights the anti-proliferative effects of the n-hexane extract on U87 (MG) cells. This study underscores the promising qualities of *Artocarpus heterophyllus* seeds and their potential in health and medicine, proposing them as a valuable source for innovative anticancer therapies and combating oxidative stress-related ailments. Incorporating these seeds into diets or therapeutic regimens could advance strategies for health promotion and disease prevention.

Keywords: *Artocarpus heterophyllus*, antioxidant activity, cytotoxicity, flavonoids, anticancer therapy, oxidative stress.

Introduction

Originating from the verdant rainforests of the Western Ghats in India, jackfruit has evolved into a culinary staple in South and Southeast Asia. This large, tropical fruit is celebrated not only for its unique taste and versatility in various dishes but also for its nutritional value, being rich in vitamins, minerals, and dietary fibre. Leading the production are regions like Tripura, Assam, and West Bengal, with Tripura standing out as the primary contributor, offering jackfruits year-round due to its favourable climate and dedicated agricultural practices. Beyond its culinary significance, the jackfruit tree is highly valued for

its multifaceted uses. The jackfruit's bark is known for its astringent and antimicrobial properties, often used in treating skin ailments and as a natural remedy for wounds. The roots are traditionally used to manage diarrhoea and fever. The seeds, which are edible when cooked, are not only a source of protein but also contain compounds with antioxidant and anti-inflammatory effects. The leaves are used in folk medicine to address diabetes and high blood pressure, showcasing the plant's wide range of medicinal applications (Elevitch and Manner, 2006; Das and Saha, 2020). Within the *Artocarpus* genus, to which jackfruit belongs, lies a reservoir of health benefits. Phytochemicals extracted from *Artocarpus heterophyllus* seeds showcase anti-inflammatory, anticancer, chemopreventive, and antioxidant attributes (Morrison *et al*, 2021). A variety of therapeutic applications can be achieved using compounds such as artocarpin,

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isoartocarpin, and norartocarpetin. These compounds can be helpful in treating fever, wounds, skin conditions, and even act as a remedy for snake bites (Prakash *et al.*, 2009).

In particular, Jackfruit seeds are often underestimated, despite their rich nutrient profile, potential health benefits, and unique taste (Hossain *et al.*, 2014). The antimicrobial, anti-inflammatory, anticancer, and antioxidant properties of these foods are attributed to their abundance in protein, fiber, and healthy fats (Shanmugapriya *et al.*, 2011). Research suggests they can mitigate the threat of chronic diseases such as cancer, diabetes, and heart disease (Sreeja *et al.*, 2021; Burci *et al.*, 2019). Given the global health challenge posed by cancer, the pursuit of effective and safer anticancer therapeutics is paramount (Gupta *et al.*, 2010). Jackfruit seeds, housing bioactive substances like phenolic compounds, flavonoids, and saponins, showcase antioxidant activity, cytotoxic effects on cancerous cells, and antiproliferative properties (Burci *et al.*, 2019; Sirla *et al.*, 2020). This positions them as a promising reservoir for the development of novel anticancer interventions.

Aligned with the objectives of ongoing research, this study endeavors to explore the binding potential of chemical constituents from *Artocarpus heterophyllus* seeds against human tubulin, scrutinize the phytochemical composition, assess antioxidant properties, and examine the potential impact of extracts obtained from jackfruit seeds on cancer cell proliferation. This research holds the promise of unveiling valuable insights for cancer prevention and treatment strategies.

Materials and Methods

All solvents and chemicals were of analytical grade and sourced from local suppliers. This included methanol, dimethyl sulfoxide (DMSO), polysorbate, formaldehyde, sodium chloride (NaCl), potassium chloride (KCl), calcium chloride (CaCl₂), ascorbic acid, aluminum trichloride (AlCl₃), 1,1-diphenyl-2-picrylhydrazyl (DPPH), ferric chloride, Folin-Ciocalteu reagent, potassium phosphate (monobasic and dibasic),

sodium carbonate, MTT (3-[4,5-Dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide), ethanol, n-hexane, etc.

Extraction procedure

Artocarpus heterophyllus (jackfruit) seeds were procured from the market, washed twice with water, and the white arils (seed coat) were carefully removed. The seeds were then dried in an incubator at 40 °C for 3-4 days without removing the brown spermoderm. Subsequently, the dried seeds (79 g) were ground into a fine powder using a grinder, and this powder was used for further processing.

The extraction process involved macerating 25 gm of the powdered samples in separate beakers containing 100 ml of a mixture comprising 80 % ethanol and absolute n-hexane. Maceration was conducted with intermittent shaking for a period of five days at room temperature. Following maceration, the extracts were filtered using Whatman Grade I filter paper and concentrated using a rotary evaporator. The resulting concentrated extracts were then stored in a refrigerator for future use (Thapa *et al.*, 2016).

Molecular docking studies

A three-dimensional (3-D) structure of each compound was determined using Cresset's Flare 7.0 software. A comprehensive optimization process was conducted on these structures, resulting in the wave function's spin state being changed to a singlet during minimization. All other parameters were maintained at their default settings for optimization using conventional SCF convergence.

Enzyme utilized

Enzymes from the Protein Data Bank (PDB) were utilized in this study. The 3-D structures of proteins and the human receptor (PDB ID: ISAO) were obtained from the Protein Data Bank (<https://www.rcsb.org>). Flare 7.0 software was employed to extract water molecules, ions, and ligands from the protein molecules, isolating the specific components required for analysis.



Protein preparation wizard

An analysis of docking was conducted using the Protein Preparation Wizard to prepare the protein structure. To accomplish this, it was necessary to assign the correct bond orders to the proteins and to add hydrogen atoms. Subsequently, an empirical usefulness algorithm was employed to perform restrained minimization of the average Root-Mean-Square-Deviation (RMSD) of hydrogen atoms while keeping the heavy atoms unchanged.

Docking

An interaction grid was generated for the protein structure to facilitate the docking process. Specifically, a grid was created in the active site of chain A for the Tubulin protein (ISA0). The receptor grid for the PDB protein (ISA0) was developed using the bound inhibitor DAMA Colchicine as a reference structure to define the active site. The grid box dimensions were determined based on the bound ligand present in the protein structure, selected from the workspace. The extracted ligand underwent necessary corrections and was re-docked using the defined grid parameters. Subsequently, the Root-Mean-Square Deviation (RMSD) was calculated. Following the validation of the docking protocol as defined by (Chaudhary *et al.*, 2022) a collection of prepared ligands was docked into the active site.

Quantification of total phenolic content

The modified Folin-Ciocalteu reagent method was employed to ascertain the total phenolic content. For each crude extract, 1 mg was dissolved in 1 mL of methanol. A 10% Folin-Ciocalteu reagent solution was prepared by combining 10 mL of the reagent with 90 mL of water. Additionally, a 5% Na_2CO_3 solution was made by dissolving 3 g of Na_2CO_3 in 50 mL of water. To conduct the assay, 200 μL of each crude sample was combined with 1.5 mL of the 10% Folin-Ciocalteu reagent solution in test tubes. These tubes were then placed in a dark location for 5 minutes. Subsequently, 1.5 mL of the 5% Na_2CO_3 solution was added to each tube, and the solutions were thoroughly mixed by hand. The test tubes

were once again kept in the dark for 2 hours. Finally, the absorbance of each solution was determined using a UV spectrophotometer at a constant wavelength of 750 nm, following references by Shanmugapriya *et al.* (2011).

The modified Folin-Ciocalteu reagent method was utilized to create a calibration curve for Gallic acid. Initially, 3 mg of Gallic acid was dissolved in 10 mL of methanol, resulting in a concentration of 300 mg/L. This solution was then diluted by adding methanol to create a series of standard concentrations: 200, 100, 50, and 25 mg/L. The same procedure was followed for the Gallic acid standard. For determine the concentration, the absorbance of all standard solutions was measured using a UV-spectrophotometer at a specific wavelength of 750 nm.

Total flavonoid content

Amounts of flavonoids in milliliters (mg/ AlCl_3) were determined with aluminum chloride (AlCl_3) using the method described by Shanmugapriya *et al.*, (2011). An assay was conducted by mixing 0.5 mL of the seed extract with 0.5 mL of distilled water and 0.3 mL of 5% NaNO_2 . The concoction was then incubated for five minutes at 25°C. A subsequent addition of 0.3 mL of 10% AlCl_3 and 2 mL of 1M NaOH was made. Using a spectrophotometer, the optical density of the reaction blend was measured at 510 nm. The comparison was conducted using quercetin as a standard.

Determination of DPPH radical scavenging activity

The DPPH scavenging activity was assessed using a spectrophotometric method. Antioxidants donate protons to scavenge the DPPH radical, resulting in reduced DPPH and a decrease in color intensity, measured at 517 nm (Sreeja *et al.*, 2021). Extracts at concentrations ranging from 10 to 50 $\mu\text{g}/\text{mL}$ were prepared in aqueous ethanol. A 0.6 mM DPPH solution in aqueous ethanol was also prepared. 0.5 mL of DPPH solution was added to tubes containing the extracts, mixed thoroughly, and incubated in darkness for 30 minutes at room temperature. After incubation, the absorbance of each sample was measured at 517 nm using a



spectrophotometer. A control solution of aqueous ethanol and DPPH and a blank solution of aqueous ethanol without extract or DPPH were used for reference. The percentage inhibition of DPPH radicals due to the antioxidant activity of the extract was calculated using the following formula:

$$\text{Percent inhibition} =$$

$$[(A \text{ control} - A \text{ sample}) / A \text{ control}] \times 100$$

Where, A control constitute the optical density of the control solution (without extract) and A sample constitute the optical density of the sample solution (with extract).

By comparing the absorbance values, the extent of DPPH scavenging activity can be determined. A higher percentage inhibition indicates a stronger antioxidant activity of the extract.

MTT cell viability assay

The MTT Cell Viability assay followed the protocol by Nguyen *et al.*, (2017). Human glioblastoma cell lines (10,000 cells/well) were initially cultured in 96-well plates and allowed to incubate for 24 hours. After this incubation period, the cells were exposed to various extracts derived from *Artocarpus heterophyllus* seeds and further incubated for an additional 24 hours. Following incubation with the extracts, the cells were washed with phosphate-buffered saline (PBS). Then, 10µl of 5 mg/ml MTT solution was added to each well, and the plates were incubated at 37°C for 2 hours to allow formazan crystals to develop. After incubation, 100µl of dimethyl sulfoxide (DMSO) was added to each well to dissolve the formazan crystals, followed by a 15-minute incubation at room temperature. The optical density of the formazan solution was measured at 570nm using a microplate reader. Cell viability was calculated using the formula:

$$\text{Cell Viability (\%)} = (\text{Absorbance of control cells} / \text{Absorbance of treated cells}) \times 100$$

This assay provided insights into the effects of the jackfruit seed extracts on cell viability, crucial for evaluating their potential therapeutic applications.

Results

Enhanced Molecular Docking Analysis

The study aimed to determine the binding affinity of chemical compounds derived from *Artocarpus heterophyllus* seeds with human tubulin using molecular docking techniques targeting the PDB ISAO. A crucial initial step involved establishing and validating a robust docking protocol. The protocol's reliability was assessed by re-docking the extracted ligand from the X-ray crystallographic protein structure using Flare 7.0, which accurately reproduced the original ligand's binding mode. For the molecular docking experiments, DAMA Colchicine served as a reference compound for evaluating three distinct chemical compounds from *Artocarpus heterophyllus* seeds for their inhibitory potential against human tubulin. Results from the screening (Table 1) indicated that all three compounds outperformed the reference based on their docking scores, suggesting superior binding capabilities and potential efficacy as inhibitors.

Among these compounds, Kaempferol D stood out with an exceptional docking score of -7.952 against the human tubulin protein. Detailed investigation revealed its interaction with key amino acid residues within the tubulin protein's active site, including THR at A179, VAL at A181, MET at B259, VAL at B315, ALA at B316, ASN at B258, LYS at B352, LEU at B255, and LYS at B255.

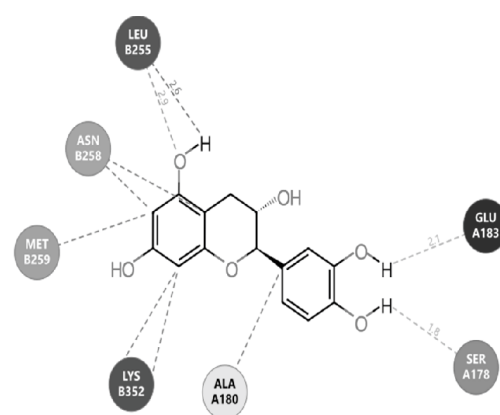


Fig. 1 : Binding model and interaction of Catechin with human tubulin protein (1SA0)

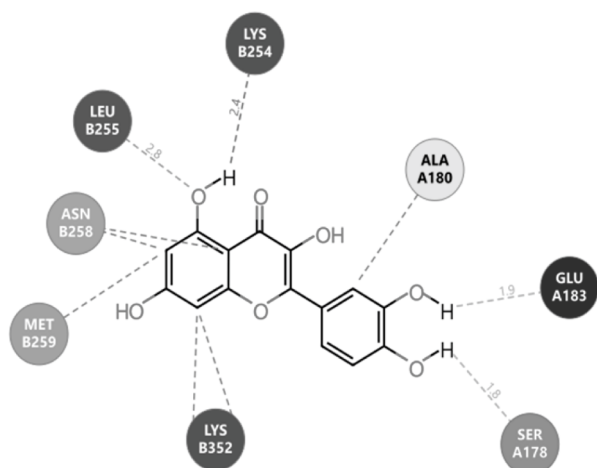


Fig. 2 : Binding model and interaction of Quercetin with human tubulin protein (1SA0)

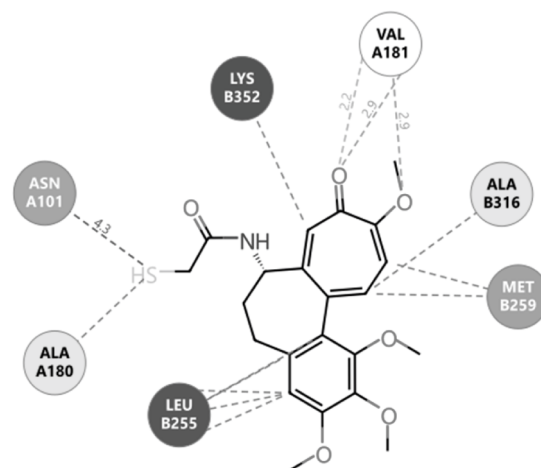


Fig. 3 : Binding model and interactions of DAMA Colchicine with human tubulin protein (1SA0)

Table 1 : In silico screening results of the phytochemicals from *Artocarpus heterophyllus* against human tubulin protein

Sr no.	Structure	Title	Protein	M.W	Atoms	Flexibility	LF.Rank score	LF DG	LF VS score
1.		Catechin D	1SA0	290.3	21	3.6	-7.938	-7.581	-8.464
2.		Kaempferol D	1SA0	286.2	21	2.2	-8.738	-7.952	-8.812
3.		Quercetin D	1SA0	302.2	22	2.7	-8.718	-7.587	-8.571
4.		DAMA Colchicine	1SA0	431.5	30	5.6	-11.451	-11.451	-12.053

Overall, these findings highlight the promising binding capabilities of chemical compounds from *Artocarpus heterophyllus* seeds against human tubulin, suggesting their potential as potent inhibitors. Detailed docking scores and interaction analyses are presented in Table 1 and Figures 4-7.

Determination of Total Phenolic Content

Various plant extracts exhibit antioxidant properties that are directly influenced by phenolic compounds, which play a pivotal role in exerting their antioxidative effects. Using different

solvents in the estimation of the phenolic content of *Artocarpus heterophyllus* seed extract at a concentration of 100 mg/g, we found that the highest concentration was found in the n-hexane extract, followed by the ethanolic extract, with a concentration of 3.745 mg/g and 0.157 mg/g, expressed as mg GAE/100g (Fig. 8).

Assessment of total flavonoid content

Analysis of two different seed extracts of *Artocarpus heterophyllus* revealed distinct levels of flavonoid content. The n-hexane extract demonstrated a significantly higher

concentration (15.126 ± 0.066 mg/g) compared to the ethanol extract (3.456 ± 0.116 mg/g), as shown in Table 2. These values are expressed as quercetin

equivalents per 100 mg of seed extract (Graph 2), highlighting the differential extraction efficiency of flavonoids from the seeds.

Table-2 : Total phenolic and flavonoid content, reducing potential of seeds extracts of *Artocarpus heterophyllus*

Different Fraction	Total Phenolic Content	Total flavonoid Test
Ethanol	3.745 ± 0.157	3.456 ± 0.116
n-Hexane	4.088 ± 0.140	15.126 ± 0.066

Assessment DPPH radical scavenging assay

The DPPH system serves as a dependable method for generating stable radicals, making it a popular choice for assessing the antioxidant capabilities of compounds. In this context, the DPPH assay was employed to measure the potential of antioxidants to neutralize free radicals. The efficacy of DPPH in capturing these radicals lies in the delocalization of the unpaired electron throughout its molecular structure.

The extract derived from *Artocarpus heterophyllus* seeds demonstrated antioxidant activity compared to the standard reference, such as ascorbic acid across various tested concentrations (20, 50, and 100 $\mu\text{g/mL}$). The antioxidant potential exhibited a gradual increase with higher concentrations for all tested levels. In this evaluation of antioxidant activity utilizing the DPPH method, ascorbic acid was employed as the reference compound. The level of ascorbic acid tested ranged from 20 to 100 $\mu\text{g/mL}$. Notably, ascorbic acid displayed an inhibition percentage of 62.64297% at 20 $\mu\text{g/mL}$ and reached 100.279% at 100 $\mu\text{g/mL}$ Table 3; Fig. 10. The calculated IC_{50} value for ascorbic acid was 3.4514 $\mu\text{g/mL}$. Furthermore, the IC_{50} values for the ethanolic and n-hexane extracts were determined as 80 $\mu\text{g/mL}$ and 35 $\mu\text{g/mL}$, respectively. The observed results indicate that the ethanolic extract exhibited a comparatively lower IC_{50} value than the n-hexane extract, suggesting a potentially stronger antioxidant effect.

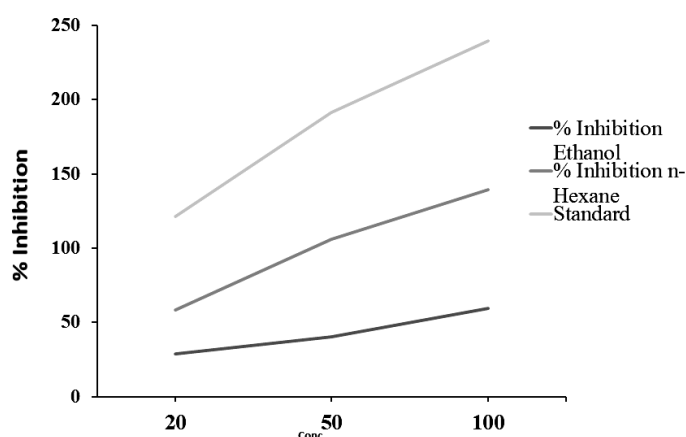


Fig. 4 : Results of DPPH assay showing % inhibition with respect to ascorbic acid

Table-3: Results of DPPH assay showing % inhibition with respect to ascorbic acid, ethanolic and n-hexane seed extracts of *Artocarpus heterophyllus*

Different concentration	Ethanol	n-Hexane	Standard
20mg	28.90788	29.73306	62.64297
50mg	40.29827	65.46152	85.61948
100mg	59.30138	79.87402	100.279

Anticancer Activity in Human Glioblastoma Cell Line (MTT Assay)

U87 (MG) cells were seeded in a 96-well plate at a concentration of 10,000 cells per well using DMEM media supplemented with 10% FBS and 1% Penicillin-Streptomycin. The plate was then incubated at 37°C in a CO₂ incubator (with 5% CO₂ and 95% RH). The test material was dissolved in Dimethyl Sulfoxide (DMSO) before application on cells (Graph no.11) (Table no.4). The test

material was tested at a concentration of 100 $\mu\text{g}/\text{well}$ with an untreated control for a 24-hour incubation period.

Subsequently, MTT solution was prepared from a 5 mg/ml stock solution in fresh DMEM media with 10 $\mu\text{L}/\text{well}/100\mu\text{L}$ calculation, and after the 24-hour incubation period, the drug was replaced with MTT added media (Figure 12). The plate was incubated at 37°C in the CO₂ incubator for the next 3 hours, and then the MTT added media was replaced with DMSO (100 $\mu\text{L}/\text{well}$) to dissolve the formazan crystals (purple). The plate was further incubated at room temperature for 30 minutes wrapped with aluminum foil. The absorbance was observed and recorded at 570 nm using the Multiskan Sky Microplate Spectrophotometer

(ThermoScientific).

The outcomes obtained from the MTT assay demonstrate that the solutions obtained by dissolving the ethanol and n-hexane extracts sourced from the seeds of *Artocarpus heterophyllus* exhibited pronounced anti-proliferation effects. Notably, at a concentration of 100 $\mu\text{g}/\text{ml}$ per well, the n-hexane solution displayed significantly elevated anti-proliferative activity, as evidenced by a viability rate of 74.58%. Additionally, ethanol showed less antiproliferation (86.75%). This noteworthy result highlights the extract's potential to curb cell growth in an in vitro cytotoxicity investigation when contrasted with the untreated control group.

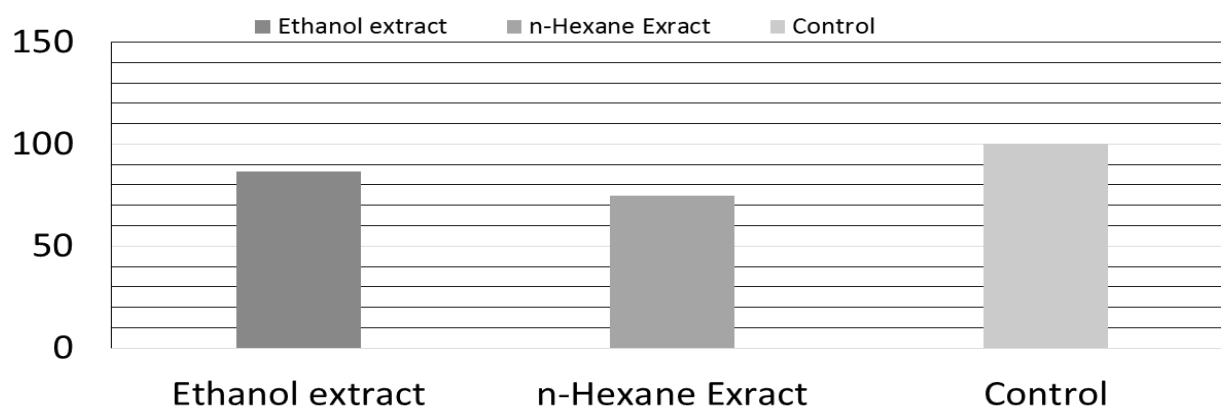


Fig. 5 : Results of MTT assay in Graphical representation of Effect of the different concentration (ethanolic extract), (n-hexanic extract) of *Artocarpus heterophyllus* seeds in U87 (Mg cells)

Table 4: Effect of the different concentration (ethanolic extract), (n-hexanoic extract) on seeds of *Artocarpus heterophyllus* in U87(Mg cells)

	Ethanolic Extract	n- Hexane Extract	Control
% Proliferation	86.7555	74.5875	100

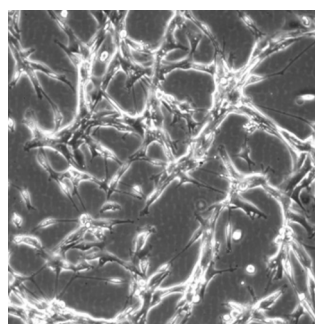
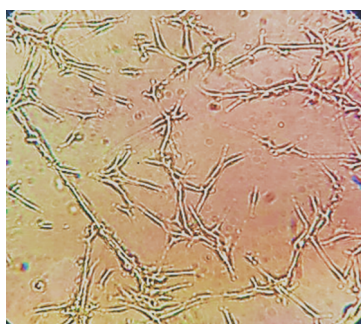


Fig. 6: U87 (MG) cell line in MTT assay

Discussion

Leveraging molecular docking in the present study has thrown light on the interactions arising between the human tubulin protein and chemical components from *Artocarpus heterophyllus* seeds. A potent computational approach in the drug development process, molecular docking offers a prediction framework for investigating the binding processes among ligand and targeted proteins. The design and optimisation of possible therapeutic options can be guided by the results of this research. The docking methodology was successfully validated, as indicated by the precise replication of the extracted ligand's binding mode, demonstrating the dependability of this approach. This stage makes sure that the procedure that has been chosen can correctly capture the interacting conformation of ligands inside the active site of the protein. The chemical components from the seed of *Artocarpus heterophyllus* plants and the reference molecule DAMA Colchicine's docking scores indicate that these compounds may have the ability to inhibit human tubulin. Kaempferol D, one of the components that underwent screening, had the best interaction result against human tubulin, demonstrating an exceptionally high affinity towards the protein's considerably binding site and pointing to a possible role as an inhibitor. The in-depth examination of the chemical reactions between tubulin's active site residues and kaempferol D sheds light on the molecular processes that underlie these interactions. These interactions stabilise the ligand inside the binding site and include hydrophobic interactions, some hydrogen bonds, and other non-covalent forces. In addition to offering insightful details about the binding mechanisms, the molecular docking studies lay the groundwork for additional experimental confirmation. Prioritising the compounds found through docking can help with manufacturing and testing to confirm that they have the ability to inhibit human tubulin in a lab environment. Furthermore, the understanding of the structural characteristics required for robust binding provided by molecular docking can direct the alteration of ligand molecules to

improve their binding affinity. Nonetheless, it is critical to recognize the limits of molecular docking research. The quality of the input structures, such as ligand and protein structure, has a major impact on the accuracy of the docking findings. The findings' dependability is also influenced by the selection of scoring functions. Moreover, protein-ligand interactions in real-world settings are dynamic, whereas molecular docking provides a static picture of binding interactions. Consequently, in order to validate the predictions generated by molecular docking, experimental methods such as cell-based assays and binding assays are essential. Molecular docking studies have provided valuable insights into the potential binding interactions between chemical constituents from *Artocarpus heterophyllus* seeds and human tubulin protein. The computational insights from docking results lay the groundwork for further experimental exploration and validation, contributing to a broader understanding of the bioactive potential of *Artocarpus heterophyllus* seeds and their applications in drug discovery and development (Chaudhary *et al.*, 2022). Phenolic compounds are noteworthy for their potent chain-breaking antioxidant properties and ability to terminate free radicals (Shahidi *et al.*, 1992). These compounds, with their hydroxyl groups, effectively scavenge free radicals (Hatano *et al.*, 1989). Flavonoids, a subgroup of phenolic compounds, act through scavenging or chelating processes (Cook *et al.*, 1996; Kessler *et al.*, 2003). Extracts with high antioxidant activity often have high phenolic content. Among the various polyphenols found in plants, phenolic compounds are distinguished by their exceptional redox properties, enabling them to efficiently absorb and neutralize free radicals. They are also capable of quenching singlet and triplet oxygen or decomposing peroxides (Zheng *et al.*, 2001). These properties confer excellent antioxidant, anti-mutagenic, and anti-cancer activities to plant-derived phenols. The phenolic compounds present in plant extracts, such as the n-hexane extract of *Artocarpus heterophyllus* seeds, exhibit remarkable antioxidative capabilities.



Their ability to neutralize free radicals through various mechanisms enhances the overall antioxidant potential of these extracts. The presence of polyphenols further amplifies their efficacy in combating oxidative stress and promoting health, making them valuable natural compounds with promising applications in various fields. Our study found that the n-hexane extract exhibited higher antioxidant potential compared to the ethanol extract of *Artocarpus heterophyllus* seeds, aligning with previous observations (Shanmugapriya *et al.*, 2011). Flavonoids, a vast group of more than 4,000 polyphenolic compounds, are ubiquitous in plant-based foods and play a significant role in human health due to their pharmacological activities as radical scavengers (Yao *et al.*, 2004). These compounds are noted for their broad range of chemical and biological properties, including antioxidant and free radical scavenging capabilities, making them one of the most important natural phenolics (Kahkonen *et al.*, 1999). Studies have highlighted flavonoids as potent antioxidants, capable of scavenging various reactive oxygen species and inhibiting lipid peroxidation. These compounds also hold potential as therapeutic agents against a diverse array of diseases (Ross *et al.*, 2002; Williams *et al.*, 2004). Phenols and polyphenolic compounds, including flavonoids, are commonly found in plant-derived food products and are known for their substantial antioxidant activity. The relationship between total phenol content and antioxidant activity has been extensively studied, particularly in fruits and vegetables (Acker *et al.*, 1996). The analysis of total flavonoid content in different *Artocarpus heterophyllus* seed extracts underscores the significance of these compounds in plant-based foods. Their abundant presence in human diets and their remarkable pharmacological properties as radical scavengers highlight their potential health benefits. The diverse chemical and biological activities of flavonoids, including their antioxidant and free radical scavenging properties, further emphasize their importance in promoting well-being. Additionally, their potential as therapeutic agents

against various diseases adds to their significance in the realm of natural phenolics. Our experimental findings showed a notable difference in total flavonoid content (15.126 and 3.456) in n-hexane and ethanol extracts, respectively, with the ethanol extract findings consistent with previous studies (Shanmugapriya *et al.*, 2011). Significant free radical scavenging effects were observed in a concentration-dependent manner in this study on *Artocarpus heterophyllus* seed extracts, particularly up to 100 µg/ml. The IC₅₀ values, representing the concentration required to scavenge 50% of DPPH radicals, were 80 µg/ml for the ethanolic extract and 35 µg/ml for the n-hexane extract. The versatility of DPPH radicals as a testing agent makes it widely used for evaluating the antioxidant activity of various compounds, including plant extracts and foods. In this study, the ethanolic and n-hexane extracts of *Artocarpus heterophyllus* seeds exhibited strong antioxidative activity, with percentages of 59.30138% and 79.87402%, respectively, at their respective concentrations of 100 µg/ml. The n-hexane extract displayed particularly significant antioxidant activity among the *Artocarpus heterophyllus* seed extracts. The observed radical scavenging activity of the plant extracts is likely due to their phenolic nature, enabling them to participate in electron transfer and hydrogen donation processes (Soares *et al.*, 1997; Porto *et al.*, 2000). Overall, the results indicate a dose-dependent relationship between the concentration of the extracts and their ability to scavenge DPPH radicals, highlighting the potential health benefits of these natural compounds as antioxidants. Our findings showed a remarkable difference in antioxidative activity (79.87402 and 59.30138) at 100 µg in n-hexane and ethanol extracts, respectively, with the ethanol extract findings consistent with previous studies (Devi *et al.*, 2021). The MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay is a robust and widely used method for evaluating cell viability and cytotoxicity. In this study, the MTT assay assessed the impact of a test material derived from *Artocarpus heterophyllus* seeds on U87 (MG) cells.



The MTT assay is based on the ability of viable cells to reduce the MTT reagent, forming formazan crystals proportional to the number of viable cells (Rodríguez *et al.*, 2021). In our study, the reduction of MTT by cells was used as an indicator of cell viability after exposure to the test material. The MTT assay results revealed significant anti-proliferative effects of the n-hexane extract from *Artocarpus heterophyllus* seeds. The n-hexane solution showed a pronounced decrease in cell viability, suggesting potential cytotoxic activity. The viability rate of 74.58% at a concentration of 100 µg/ml indicated a substantial reduction in viable cells. In contrast, the ethanol extract displayed a lesser impact on cell viability, with a viability rate of 86.75%. These findings underscore the differential cytotoxic potential of the two extracts and emphasize the need for further investigation into their bioactive constituents. It's important to recognize that the MTT assay, while valuable, provides a snapshot of cell viability under specific conditions. The results are influenced by various factors, such as the characteristics of the cell line, the experimental setup, and potential interactions between the test material and the cells. Further studies involving additional concentrations, exposure times, and complementary assays are necessary to comprehensively assess the cytotoxic effects and elucidate the underlying mechanism. The MTT assay presented a reliable means to evaluate the cytotoxic effects of the test material derived from *Artocarpus heterophyllus* seeds on U87 (MG) cells. The assay's simplicity and sensitivity make it a cornerstone in cell viability assessments, aiding researchers in understanding the potential impacts of various agents on cellular health. Nonetheless, careful interpretation and consideration of experimental variables are essential for deriving meaningful insights from MTT assay results (Patel and Patel, 2011; Burci *et al.*, 2019). The observed results of the MTT assay demonstrated that the n-hexane extract of *Artocarpus heterophyllus* seeds exerted significant anti-proliferative effects on U87 (MG) cells. The n-hexane extract exhibited a concentration-dependent reduction in cell viability, with a

pronounced impact observed at a concentration of 100 µg/ml. This effect is indicative of the extract's cytotoxic potential against these glioblastoma cells. In contrast, the ethanol extract displayed a milder reduction in cell viability, suggesting a less potent cytotoxic effect. The differential cytotoxic effects observed between the two extracts underscore the potential importance of the extraction solvent in determining the bioactive constituents present in the extracts. The n-hexane extract, which exhibited more pronounced cytotoxicity, may contain compounds with stronger anti-proliferative properties. However, further studies are necessary to identify the specific bioactive compounds responsible for the observed effects and to decipher their underlying mechanisms of action.

Conclusion

This study conducted on the *Artocarpus heterophyllus* (Jackfruit) seed extracts has provided valuable insights into its potential bioactive properties. The research encompassed various experimental techniques, ranging from molecular docking assessment to assess binding potential, to the exploration of antioxidant and cytotoxic activities through in vitro assays. The molecular docking studies demonstrated that chemical constituents present in seeds of *Artocarpus heterophyllus* exhibited promising binding capabilities against human tubulin protein. These findings suggest the potential of these substances as effective inhibitors, potentially contributing to their role in drug discovery and development. The evaluation of total phenolic and flavonoid content revealed the presence of these bioactive compounds in different seed extracts. The n-hexane extract demonstrated higher level of phenolic and flavonoid content compared to the ethanol extract. These phenolic compounds are known for their antioxidative properties, which contribute to their potential health benefits as free radical scavengers. The DPPH radical scavenging assay further substantiated the antioxidant potential of *Artocarpus heterophyllus* seed extracts. Both ethanol and n-hexane extracts exhibited notable



radical scavenging activity, with the n-hexane extract displaying a more potent effect. These results underline the extracts' potential as natural sources of antioxidants. In the cytotoxicity evaluation using the MTT assay, the n-hexane extract displayed significant anti-proliferative effects on U87 (MG) cells. The reduced cell viability observed suggests potential cytotoxic activity. On the other hand, the ethanol extract exhibited a lesser impact on cell viability, indicating a differential effect between the two extracts. This comprehensive study sheds light on the potential bioactivity of *Artocarpus heterophyllus* seed extracts. The observed binding potential, antioxidant activity, and cytotoxic effects indicate the presence of valuable bioactive compounds in the seeds. These findings offer a foundation for further research, potentially leading to the development of novel pharmaceutical agents or functional food ingredients. However, further investigations are necessary to elucidate the specific bioactive compounds responsible for these effects and to uncover the underlying mechanisms of action.

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Investigation of Seed Germination Stages in *Hordeum vulgare* L. Under Salt Stress

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Abstract

Salinity is one of the most significant environmental factors that has unfavourable impacts on the growth of crop plants. It has been estimated that salt stress affects ~2.1% of dry land and ~19.5% of all irrigated land and these percentages continue to increase. The present research work aims to evaluate the effect of NaCl on barley during the seed germination stage. We determined different attributes of barley seeds during the germination stage. The research work integrated germination percentage (G%), germination index (GI), mean germination time (MGT), mean germination rate (MGR), coefficient of variation of germination time (CV_t), coefficient of velocity of germination (CVG) and germination rate index (GRI). Statistical analysis was carried out by a one-way analysis of variance (ANOVA). Different concentrations of NaCl (50 mM, 100 mM, 150 mM and 200 mM) were used to study the impact of salinity on seed germination. The present investigation revealed that different concentrations of NaCl (50 mM, 100 mM, 150 mM and 200 mM) significantly affected the seed germination parameters of barley crops. The germination percentage decreased with increasing concentration of NaCl solution. The concentration of ≥150 mM of NaCl in the soil significantly inhibited seed germination. The GI also decreased with increasing concentrations of NaCl. MGT of barley seeds significantly increased with increasing concentration of NaCl in the soil. On the other hand, other seed germination parameters, viz., MGR, CV_t, CVG and GRI, also exhibited a significantly declining trend with increasing concentration of NaCl. It can be concluded that this negative effect of NaCl on barley seeds is dose-dependent.

Keywords: Salinity stress, NaCl, Barley, Seed germination.

Introduction

Salt stress is the most noteworthy abiotic factor that has a damaging impact on crop productivity in terms of quantity and quality around the world (Hafsi *et al.*, 2010; Amin *et al.*, 2021; Gupta *et al.*, 2021). It has been estimated that salt stress affects ~2.1% of dry land and ~19.5% of all irrigated land (FAO 2005). The degree of saline regions is still growing as a result of improper irrigation practices (Jamil *et al.*, 2011; Rasheed *et al.*, 2020). The disproportionate environmental harnessing, climate change and global warming have also

become major threats, adversely affecting plant productivity (Sadiq *et al.*, 2020). Furthermore, the salinisation process takes place in dry and semi-arid environments because of excessive evaporation and insufficient precipitation to allow for significant leaching (Dai *et al.*, 2011). Salinity could decrease or completely stop seed germination processes due to the accumulation of Na⁺ and Cl⁻ ions in plant tissue subjected to soil systems with high concentrations of NaCl (Maathuis *et al.*, 2014; Kshetrimayum *et al.*, 2017; Debez *et al.*, 2020). A high concentration of sodium (Na⁺) ions inhibits the uptake of potassium (K⁺) ions, which is one of the most important elements for plant growth (Kronzucker *et al.*, 2013; Gupta and Huang 2014).

Plants have developed various physiological and biochemical mechanisms to maintain a relatively

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stable intracellular environment via accumulating various solutes under saline conditions (Gupta and Huang 2014; Roy *et al.*, 2014). Furthermore, complex characteristics such as osmotic stress, ion toxicity, mineral deficiencies, and physiological and biochemical abnormalities are some of the ways that salinity hinders crop growth and development (Puyang *et al.*, 2015; Xu *et al.*, 2016; Lin *et al.*, 2017; Ali *et al.*, 2021; Johnson and Puthur, 2021). Moreover, reactive oxygen species (ROS) are produced in mitochondria, apoplastic space and chloroplasts under salinized circumstances as a result of osmotic and ionic stress (Nazar *et al.*, 2011; Khan *et al.*, 2012; Hasanuzzaman *et al.*, 2020). The quality and overall yield of the impacted crop are eventually decreased by this oxidative stress, which also damages nucleic acids, cell membranes and cellular structure. Membrane peroxidation and ion leakage are further effects (Hessini *et al.*, 2015). It may also negatively impact the structure of the soil and the quality of the water (Dehnavi *et al.*, 2020). Osmotic and specific-ion effects are the main ways in which high concentrations of soluble salts, including sodium chloride (NaCl), influence germination (Huang and Redmann, 1995; Rengasamy, 2016; Hussain *et al.*, 2019). Because of ionic pressure, high salt levels in the soil can impact plant growth and development. The absorption of vital nutrients is impacted when the osmotic potential of the soil solution decreases (Dhiman *et al.*, 2021).

The present study aimed to investigate the effect of different concentrations of NaCl on the seed germination attributes of barley crops.

Methodology

Certified seeds of barley (*Hordeum vulgare* L. variety RD2035) were procured from the Indian Agricultural Research Institute, Pusa, New Delhi. Seeds were surface sterilized in 2% sodium hypochlorite and then washed with double distilled water and used for pot experiments.

Pot experiments

Germination analysis was conducted with germinating barley irrigation by different concentrations of NaCl from November 2020 to

December 2020 in the Botany Department, Chaudhary Charan Singh University (CCSU), Meerut, Campus. The soil was collected from the agricultural land near CCSU, Meerut and subjected to oven-dried followed by autoclaving. The pots were maintained in the iron-netted greenhouse. To study the salinity effect, different concentrations of NaCl (50 mM, 100 mM, 150 mM and 200 mM) were used in this study. A total of fifteen pots (three each for control, 50mM, 100 mM, 150 mM and 200 mM) were prepared and maintained in the iron-netted natural light supply greenhouses. 3.0 kg of soil was put in each plastic pot for the present investigation. An equal amount (250 ml) of different concentrations of NaCl and double-distilled water (for control) was added to each prepared pot to soak the soil and on next day, ten viable seeds of the barley crop were sown at a depth of 2-4 cm in each pot. The number of seeds germinated was recorded daily for up to 18 days. From these germination counts, several germination attributes such as germination percentage (G%), germination index (GI), mean germination time (MGT), mean germination rate (MGR), coefficient of variation of germination time (CV_t), coefficient of velocity (CVG) and germination rate index were calculated according to Ranal *et al.* (2009).

Statistical analysis

The data on germination attributes were subjected to one-way ANOVA at 0.05 significance level, and the differences among means were evaluated by the test of Tukey at 0.05 significance level. The statistical analyses were done with the SPSS 20.0 software, and graphical analysis was carried out using MS Excel 2010.

Results

The present investigation revealed that different concentrations of NaCl (50, 100, 150 and 200 mM) significantly affected the seed germination parameters of barley crops (Figure 1). The germination percentage decreased with increasing concentration of NaCl solution. However, it was noticed that the concentration of ≥ 150 mM of NaCl in the soil significantly inhibited seed germination (Figure 1a). The germination



index also decreased with increasing concentrations of NaCl solution (Figure 1b). However, the mean germination time (MGT) of barley seeds significantly ($p < 0.05$) increased with increasing concentration of NaCl in the soil (Figure 1c). On the other hand, various estimated seed germination parameters, *viz.*, mean germination rate, coefficient of variation of germination time, coefficient of velocity of germination and germination rate index, observed a significantly declining trend with increasing concentration of NaCl. CVG, GRI and MGT are well-known three indices that assess the germination rate of seed (Panuccio *et al.*, 2014). It was shown that higher CVG and GRI and lower MGT represent higher and faster germination of seed (Panuccio *et al.*, 2014). Our results indicated that a higher concentration of NaCl remarkably inhibited seed germination and delayed the seed germination time in barley seeds. Similar results

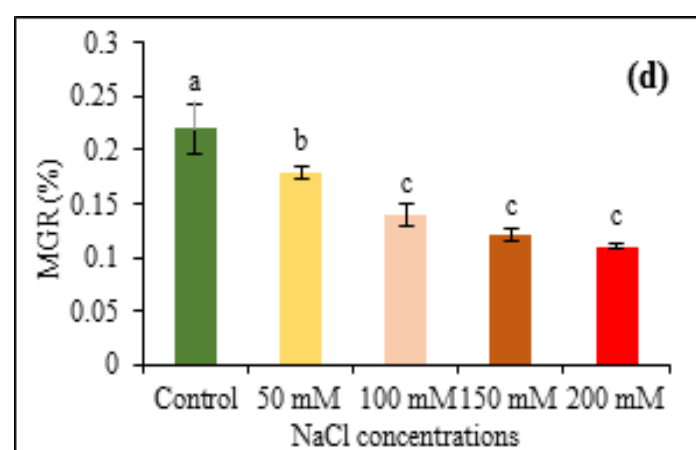
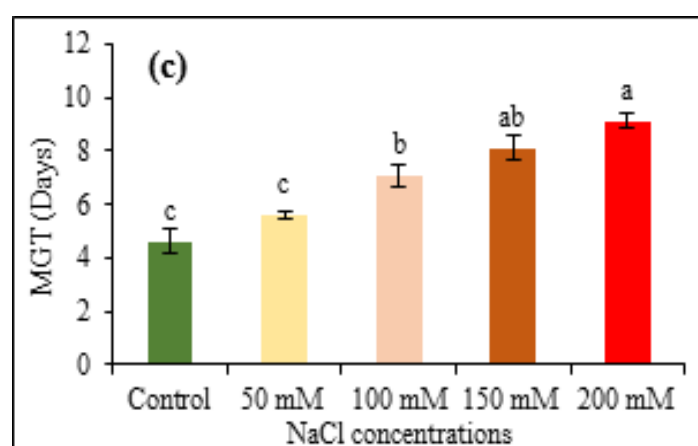
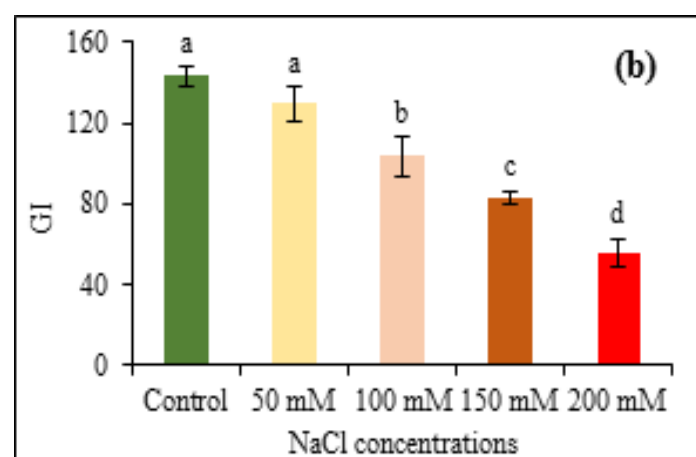
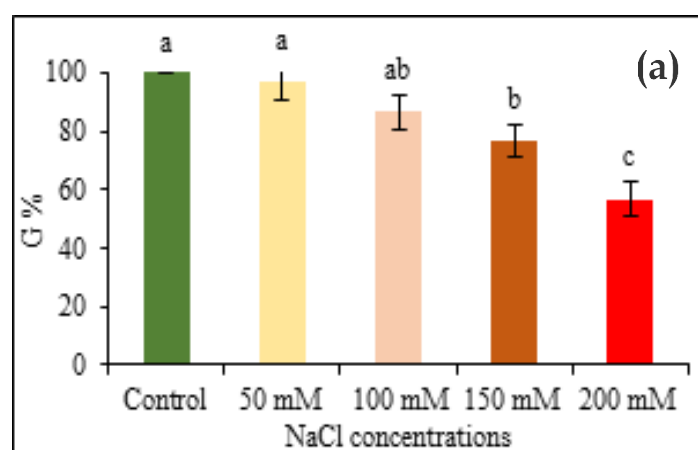
were recorded in other crop seeds such as rice (Xu *et al.*, 2011), wheat (Akbarimoghaddam *et al.*, 2011), maize (Khodarahmpour *et al.*, 2012) and sunflower (Wu *et al.*, 2015). The negative impacts of salinity may be due to ion toxicity on seed germination, as a consequence of a coincident increase in anion and cation (Panuccio *et al.*, 2014).

Conclusion

The findings of this study revealed that the high concentrations of NaCl inhibited the seed germination and delayed the germination time of *Hordeum vulgare* L. This negative effect of NaCl on barley seed is dose-dependent.

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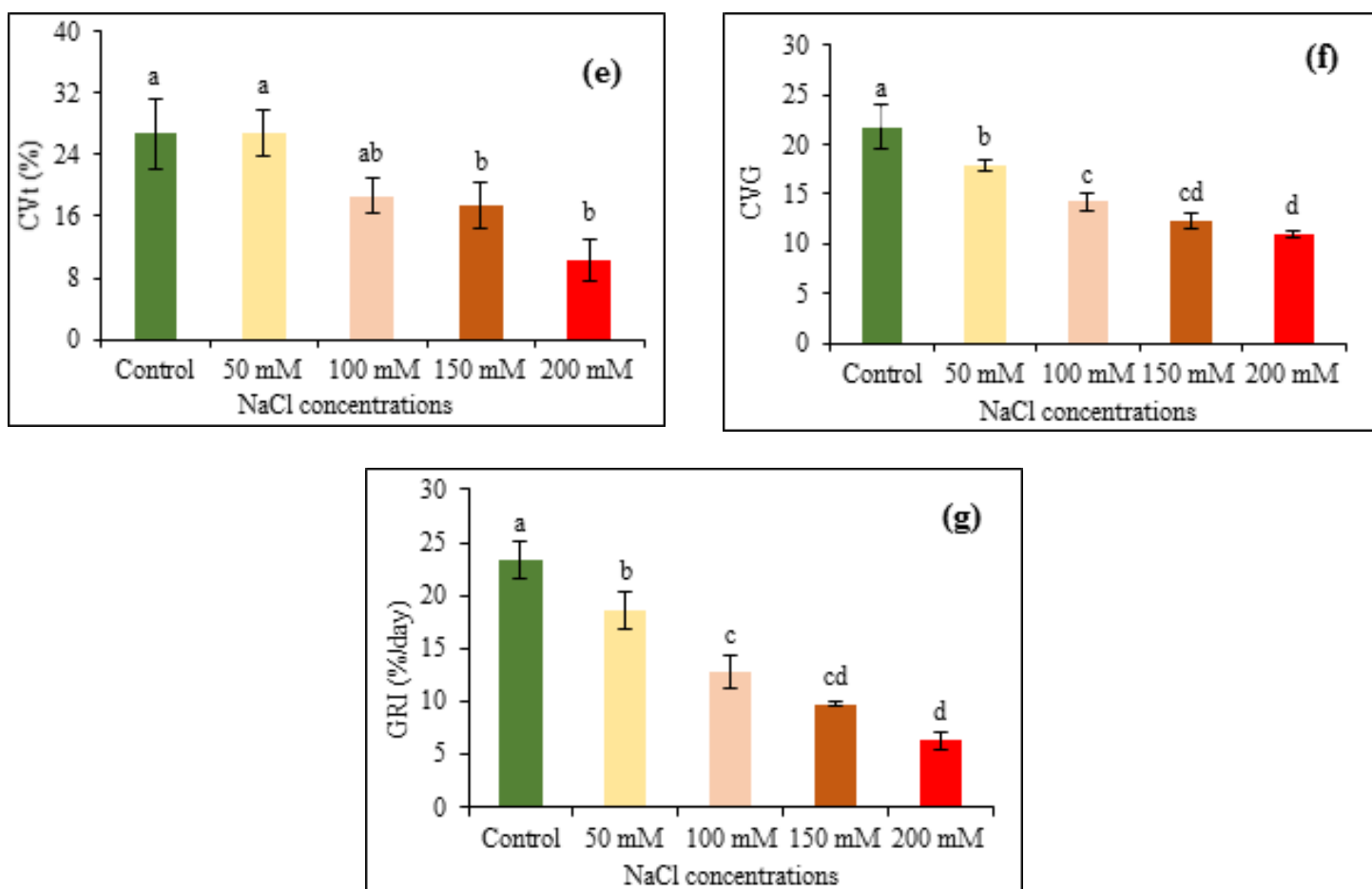


Fig. 1 : Effect of different concentrations of NaCl on the seed germination attributes (a. germination percentage (G%), b. germination index (GI), c. mean germination time (MGT), d. mean germination rate (MGR), e. coefficient of variation of germination time (CVt), f. coefficient of velocity (CVG) and g. germination rate index (GRI) of barley (*Hordeum vulgare* L.). The same letter/s over the bars do not differ from each other, employing ANOVA compared to the Tukey's test at a 0.05 significance level.

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Climate-Smart and Regenerative Agriculture Using Biotechnology with AI, IoT and Remote Sensing

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Abstract

Agriculture is undergoing a paradigm shift in response to the dual crises of climate change and ecosystem degradation. Climate-smart and regenerative agriculture, grounded in the principles of sustainability, resilience and resource efficiency, offers a transformative framework to meet global food security demands while mitigating climate impacts. The integration of biotechnology including advanced genomic tools, microbial engineering and biofertilizers with digital technologies such as Artificial Intelligence (AI), Internet of Things (IoT) and remote sensing is driving unprecedented innovation in agricultural systems. This review synthesizes recent peer-reviewed literature to present a consolidated understanding of how these technologies are jointly enabling precision interventions, enhancing soil health, increasing biodiversity and promoting carbon sequestration. The paper evaluates global and Indian case studies, examines socio-economic outcomes and highlights future research avenues, emphasizing how the convergence of these tools can operationalize truly climate-positive farming practices by 2040.

Keywords: Climate-smart agriculture, regenerative agriculture, biotechnology, AI, IoT, remote sensing, CRISPR, soil health, carbon sequestration

Introduction

Global agriculture faces an unprecedented pressure to deliver adequate, nutritious and affordable food to nearly 10 billion people by 2050 while addressing environmental sustainability (Godfray *et al.*, 2010). Current production systems contribute approximately 21–37% of global greenhouse gas emissions (IPCC, 2019) and are highly vulnerable to prolonged droughts, erratic rainfall, soil salinization and biodiversity loss. In this context, Climate-Smart Agriculture (CSA) and Regenerative Agriculture (RA) have emerged as critical frameworks for agricultural transformation (Lal, 2020; FAO, 2022).

CSA is defined by the FAO as an approach that sustainably increases productivity, enhances resilience (adaptation), reduces greenhouse gases

and enhances achievement of national food security goals (FAO, 2022). RA, while aligned with CSA, places a stronger emphasis on improving and restoring soil health, ecosystem services and carbon storage, often going beyond net-zero targets toward net-positive environmental goals (Schreefel *et al.*, 2020). The integration of biotechnology with advanced digital technologies particularly AI, IoT and remote sensing is enabling real-time, data-driven and sustainable farming practices. This technological convergence is not only enhancing productivity but also increasing the adaptability of agro-ecosystems to climate extremes.

The Concept of Climate-Smart Agriculture (CSA)

The FAO three-pillar framework of CSA emphasizes:

- 1. Sustainable Productivity Increase–** Integrating modern inputs (e.g., improved seeds, precision fertilizers) and agro-

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ecological practices to enhance yields without degrading resources.

2. **Adaptation and Resilience** – Building systems that absorb climate shocks through stress-tolerant crops, diversified cropping systems and microclimate management.
3. **Mitigation** – Reducing GHG emissions via efficient resource use, improved livestock management and carbon sequestration.

Recent studies highlight that CSA adoption is closely linked to technology accessibility, farmer education and policy incentives (Rosenstock *et al.*, 2019). Thus, for CSA to scale globally, enabling environments that embrace biotech and digital tools must be cultivated.

Regenerative Agriculture Principles

Regenerative agriculture focuses primarily on:

- Soil Regeneration and Organic Carbon Enhancement
- Water Cycle Restoration
- Biodiversity Promotion
- Reduced Reliance on Synthetic Chemicals

Techniques include cover cropping, crop-livestock integration, minimal tillage and agroforestry (LaCanne and Lundgren, 2018). Biotechnological innovations support these practices by producing crop varieties with higher biomass production, greater nutrient-use efficiency and compatibility with low-input systems.

For example, gene-edited legumes with optimized symbiotic nitrogen fixation reduce the need for synthetic fertilizers, a major source of nitrous oxide emissions (Oldroyd and Leyser, 2020). Similarly, microbial inoculants derived from rhizosphere analysis restore degraded soils while enhancing resilience to pests and pathogens.

Biotechnology as the Backbone of Climate-Smart and Regenerative Systems

Biotechnology provides the molecular and microbial tools needed for biological intensification, allowing more output with fewer inputs.

Crop Genetic Improvement

Technologies such as **CRISPR-Cas9** and TALENs enable precise modifications for:

- Drought tolerance via manipulation of ABA signalling pathways
- Salinity tolerance through Na^+ transport regulation (Munns *et al.*, 2020)
- Heat tolerance via HSP gene regulation
- Enhanced photosynthetic efficiency (South *et al.*, 2019)

Example: In rice, CRISPR-mediated OsSWEET14 gene editing has conferred resistance against bacterial blight while maintaining yield (Oliva *et al.*, 2019).

Microbiome Engineering

Advances in meta-omics have made it possible to identify beneficial microbes and reintroduce them through biofertilizers or seed coatings, enhancing nutrient uptake and resistance to stress (Compant *et al.*, 2019).

Biopesticides and Biofertilizers

Microbial fungicides, bactericides and insecticides reduce environmental contamination. Mycorrhizal fungi biofertilizers improve phosphorus acquisition critical in low-input regenerative systems.

Digital Transformation in Agriculture and Synergy of Technologies

The global agricultural sector is rapidly evolving with the infusion of Artificial Intelligence (AI), Internet of Things (IoT) and remote sensing technologies. Collectively, these tools are reshaping how data is collected, interpreted and applied for climate-smart and regenerative farming.

Artificial Intelligence (AI)

AI has emerged as a powerful enabler of precision farming systems (Kamilaris and Prenafeta-Boldú, 2018). By leveraging machine learning models and big data analytics, AI can:

- **Predict crop stress** from drought, pests, or diseases using multi-layer data including weather forecasts, soil moisture, spectral



imagery and plant physiology.

- **Optimize farm inputs** by recommending precise fertiliser and water schedules based on real-time field conditions.
- **Support genetic improvement programs** via genomic selection algorithms that identify genes linked to multiple stress tolerances.

In recent field trials, convolutional neural networks (CNNs) trained on hyperspectral drone imagery detected early wheat rust infections with >95% accuracy before symptoms were visible to the naked eye (Singh *et al.*, 2021).

AI also supports decision support systems (DSS) that integrate biophysical modelling with sustainability metrics enabling farmers to choose interventions with the best yield-to-resource ratio and environmental performance.

Internet of Things (IoT)

The IoT ecosystem in agriculture consists of interconnected sensors, actuators and cloud-based platforms enabling seamless real-time monitoring. Common devices include:

- **Soil moisture and nutrient sensors** for irrigation and fertigation control.
- **Automated weather stations** recording temperature, rainfall, humidity and evapotranspiration.
- **Livestock wearables** tracking herd health for integrated crop-livestock systems.

IoT enables site-specific management, which matches regenerative agriculture principles of tailoring interventions to micro-sites, thereby reducing overuse of resources and improving soil health (Wolfert *et al.*, 2017).

For example, in Maharashtra, India, an IoT-enabled drip irrigation system reduced water use in pomegranate orchards by 42% while increasing yields by 22% (Kulkarni *et al.*, 2020).

Remote Sensing

Remote sensing via satellites or Unmanned Aerial Vehicles (UAVs) provides macro-level monitoring with high spatial and temporal coverage.

Key applications include:

Vegetation indices such as NDVI (Normalized Difference Vegetation Index) for biomass estimation and crop vigour assessment

Thermal imaging for canopy temperature and water stress monitoring

Radar imaging (SAR) for soil moisture detection even under cloud cover

The European Sentinel-2 satellite, for instance, can detect chlorophyll content changes linked to nutrient deficiencies, while radar data supports flood mapping for risk mitigation.

These tools allow for *landscape-level tracking* of regenerative metrics such as cover crop persistence, agroforestry canopy expansion and carbon sequestration.

Synergy of Biotechnology with AI, IoT and Remote Sensing

Individually, biotechnology, AI, IoT and remote sensing deliver incremental gains. Combined, they create a closed-loop agricultural system where biological insights drive digital decisions, which in turn feed back into biological innovation.

Workflow Illustration

1. **Biotech innovations** (e.g., drought-resistant CRISPR rice) are planted.
2. **IoT sensors**- monitor real-time soil and climate variables.
3. **AI models**- process sensor and satellite data, predicting optimal irrigation timing and detecting stress anomalies early.
4. **Targeted interventions**- automated drip irrigation, microbial amendments are applied.
5. **Remote sensing** - validates results and documents regenerative outcomes such as improved NDVI trends and soil carbon increase.

Benefits of Integration:

- Fewer external inputs → lower emissions and costs
- Higher yield stability under climate variability
- Enhanced documentation for carbon credit markets and sustainability certification

This integrated system is especially impactful in regenerative projects, where tracking biodiversity and soil health improvements is as critical as maximizing yield.

Case Studies

Global: Brazil's Integrated Soybean System: In Brazil's Cerrado region, researchers deployed IoT soil probes with a heat-tolerant, gene-edited soybean variety. AI-assisted irrigation scheduling reduced water use by 37%, while microbial biofertilizers increased nitrogen fixation, resulting in 15% higher yields with zero synthetic nitrogen input (Silva *et al.*, 2022).

India: Rice-Wheat Belt Precision Regeneration: In Punjab, CRISPR-based bacterial-blight-resistant rice varieties were combined with drone-based nutrient scouting and IoT-enabled irrigation. Over three years, farmers reported 18% higher yields, a 30% reduction in urea usage and measurable soil organic carbon gains (ICAR-IARI, 2023).

Environmental and Socio-economic Impacts, Challenges and Future Pathways

Environmental Benefits

The integration of biotechnology with AI, IoT and remote sensing in climate-smart and regenerative systems has demonstrable environmental benefits:

GHG Emission Reduction: Stress-tolerant crop varieties (e.g., drought-resistant sorghum) reduce irrigation requirements, lowering energy-related CO₂ emissions (FAO, 2022).

Soil Carbon Sequestration: Microbial biofertilizers and cover crops increase soil organic matter, with field data showing carbon gains of up to 1.0–1.5 t C ha⁻¹ annually under improved management (Lal, 2020).

Biodiversity Enhancement: Reduced pesticide inputs through bio-based alternatives sustain beneficial insect populations and non-target organisms (Pretty *et al.*, 2018).

Water Use Efficiency: IoT-assisted precision irrigation systems typically cut water use by 30–50% in arid regions, preserving aquifers

(Kulkarni *et al.*, 2020).

Socio-economic Outcomes

Income Stability: Diversified, resilient farming systems buffer farmers against climate shocks and market volatility (De Pinto *et al.*, 2020).

Access to Carbon Markets: Verified regenerative practices, documented via remote sensing and IoT data, open opportunities for smallholders to earn from carbon credits (Gold Standard, 2021).

Rural Employment: Adoption of digital tools creates new roles in agri-data analysis, drone operation and sensor maintenance, fostering rural tech-employment ecosystems.

Case: In Madhya Pradesh, India, female smallholders participating in a precision-biofertilizer program increased net farm income by 28%, partly due to reduced input costs and premium payments for verified sustainable produce (ICAR-IARI, 2023).

Barriers to Adoption

Despite the promise, several challenges impede the widespread integration of biotech and digital technologies into CSA and RA frameworks:

Economic Barriers: High initial costs for IoT infrastructure and biotech seeds can deter low-income farmers.

Digital Divide: Rural regions often face poor internet connectivity, limiting real-time data transmission (Wolfert *et al.*, 2017).

Regulatory Complexity: Gene-edited crops face varied approval processes across countries, delaying deployment (Menz *et al.*, 2020).

Skills Gap: Effective use of AI and IoT tools requires training in data literacy, which is scarce in many farming communities.

Data Ownership Concerns: Farmers may be reluctant to share field data without assurance of benefit-sharing and privacy protections.

Future Pathways

The next two decades will see deeper **biological-digital convergence:**

Omics-AI Integration: Linking genomic, transcriptomic, proteomic and metabolomic data



with AI for ultra-fast breeding of climate-resilient crops (Varshney *et al.*, 2021).

Edge AI in Agriculture: Deployment of AI models locally on farm devices to enable instant decision-making without constant internet access.

Synthetic Microbial Consortia: Tailored plant-microbe partnerships for location-specific nutrient cycling and resilience.

Regenerative Robotics: Autonomous machines equipped with multispectral cameras to perform selective weeding, plant health checks and carbon monitoring.

Policy Innovations: Frameworks to integrate farmers into climate financing via verifiable impact data streams from IoT and remote sensing.

Conclusion

Climate-smart and regenerative agriculture, empowered by biotechnology and augmented with AI, IoT and remote sensing, represents one of the most viable pathways toward a sustainable and food-secure future.

By enhancing productivity, building resilience, restoring ecosystems and enabling participation in climate finance, these integrated systems respond holistically to the demands of the 21st century.

However, success depends on equitable access, farmer-centric policies, strong research-extension linkages and global cooperation in technology sharing. If these elements align, agriculture by 2040 can transition from being a driver of environmental degradation to a force for planetary regeneration.

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High-Throughput Screening of Chickpea Genotypes for Dry Root Rot Resistance Using a Blotting Paper Method and Image Analysis

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Abstract

Dry root rot (DRR), caused by the necrotrophic fungus *Macrophomina phaseolina*, is an emerging threat to chickpea (*Cicer arietinum* L.) cultivation, particularly under drought and high temperature stress. This study employed a high-throughput blotting paper method to screen five chickpea genotypes, alongside one resistant (ICC-4958) and one susceptible (BG 212) check, for DRR resistance. Seven days post-inoculation with *M. phaseolina*, root morphology was assessed using a root scanner, DSLR camera, and stereo microscope. Disease incidence, root necrosis, and microsclerotia presence were quantified. Out of the five genotypes studied, three (ICC 348528, ICC 552241, and ICC 83325) exhibited significant resistance, with minimal root necrosis and low microsclerotia counts whereas, two genotypes viz. ICC 305467 and ICC 95167 suggested partial resistance or susceptibility. ICC 348528, ICC 552241, and ICC 83325 emerge as promising candidates for breeding programs. This rapid, cost-effective screening method offers a robust tool for identifying DRR-resistant chickpea genotypes, facilitating breeding programs for improved disease resistance.

Keywords: Chickpea, Dry Root Rot, *Macrophomina phaseolina*, Blotting Paper Method, High-Throughput Screening, Root Imaging

Introduction

Chickpea (*Cicer arietinum* L.), also known as garbanzo bean, chana, Bengal gram, is a vital legume crop for global food security, providing a rich source of protein in rainfed agricultural systems. However, dry root rot (DRR), caused by *Macrophomina phaseolina* (Tassi) Goid (syn. *Rhizoctonia bataticola*), and poses a significant threat, particularly under moisture stress conditions, leading to yield losses of 50–100% in severe cases (Rai *et al.*, 2022). Among the root diseases affecting chickpea, Dry Root Rot (DRR) disease, is the most prevalent (Rai *et al.*, 2022; Mirchandani *et al.*, 2023). The disease manifests as brittle taproots, lack of lateral roots, and yellowing foliage, often exacerbated by drought. Effective screening methods are critical for

identifying resistant genotypes to support breeding programs. *M. phaseolina*, belonging to the family Botryosphaeriaceae, is a soil-borne necrotrophic fungus that causes disease in over 500 plant species. The wide host range and the ability of *M. phaseolina* to persist in the soil for extended periods as microsclerotia pose significant challenges for disease control. Quick identification of the disease is important for proper management and treatment of this disease. Traditional field-based screening for DRR is labor-intensive and influenced by environmental variability. The blotting paper method offers a rapid, cost-effective alternative for controlled in vitro screening, enabling precise assessment of plant-pathogen interactions. This study utilized a high-throughput blotting paper method to evaluate five chickpea genotypes, with BG 212 (susceptible) and JG 62 (resistant) as checks, for DRR resistance. Root morphology and disease symptoms were analyzed using a root scanner, DSLR camera, and stereo microscope on the 7th

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day post-inoculation with *M. phaseolina*. The main objectives of the study were to assess disease incidence, quantify root necrosis, and validate the blotting paper method for high-throughput DRR screening.

Materials and Methods

Plant Material: Five chickpea genotypes viz. IC-95167, IC-83325, IC-552241, IC-348528 and IC-305467 were selected based on diverse genetic backgrounds. BG 212 (DRR- susceptible) and ICC-4958 (DRR- resistant) served as checks. Seeds were surface-sterilized with 2% sodium hypochlorite for 2 minutes, rinsed with sterile water. The surface sterilized seeds were sown in polythene bags filled with tyndallized soilrite and maintained at 28°C, 16h photoperiod with a relative humidity of 60- 70% in an infection room. After seven days, the plants were uprooted and washed with tap water carefully to remove soil particles completely without damaging the roots.

Fungal Inoculum Preparation: *Macrophomina phaseolina* isolate was cultured on potato dextrose agar (PDA) at 28°C for 5 days. Inoculum was prepared by scraping mycelia and microsclerotia from PDA plates, suspending them in sterile water, and blending for 3 minutes to achieve a homogeneous suspension. Then Sub-cultured on PDA plates. 4-5 days old *Macrophomina phaseolina* culture was taken and inoculated in PDB broth with actively growing mycelium and incubated at 28°C at 180 rpm in dark during the same day of sowing. The fungal mycelia and microsclerotia

were filtered out from the suspension culture and diluted at 40% with autoclaved RO water.

Treatment with inoculum: The plant roots were dipped in the inoculum for a minute with intermittent shaking ensuring uniform inoculum load. Control seedlings were dipped in sterile water. Each genotype was tested in three replicates, with 5 seedlings per replicate. After 7 days, the inoculated plants were observed for the disease symptoms.

Blotting Paper Method

The blotting paper technique was adapted. Seven-day-old seedlings were placed on sterile blotting paper (20 × 30 cm) moistened with sterile water. Inoculated seedlings were maintained at 28°C, 16h photoperiod with a relative humidity of 60-70% for 7 days 25°C, 70% relative humidity, 12-h photoperiod.

Imaging and Data Collection

On the 7th day post-inoculation, roots were imaged using: Root image scanner (WinRhizo) for high-resolution root morphology analysis, DSLR Camera (Deluxe) for macroscopic documentation of root necrosis and discoloration and Stereo Microscope (10x objective lens of SMZ25. Scale bar = 500 µm) for microsclerotia observation, which were then used to score Disease severity index (DSI). Manual scoring was done by visual observation as given in Table 1. (Irulappan and Senthil-Kumar, 2021; Rai *et al.*, 2021).

Observation/Symptoms	Rating/Score	Disease Reaction
No infection on roots	1	Immune
Very few small lesions on roots	>1 and <3	Resistant
Lesions on roots clear but small; new roots free from infection	>3 and <5	Moderately resistant
Lesions on roots; many new roots generally free from lesions	>5 and <7	Susceptible
Roots infected and completely discoloured	>7	Highly susceptible



Result and Discussion

The disease severity index (DSI %) of five chickpea genotypes, assessed on day 7 post-inoculation with *Macrophomina phaseolina* using the blotting paper method, varied across three image acquisition methods: root scanner, stereo zoom microscope, and DSLR camera (Figures 2 and 3). The susceptible check, BG 212, consistently exhibited high DSI values across all methods, with

82% (root scanner), 85% (stereo microscope), and 82% (DSLR camera), confirming its vulnerability to dry root rot (DRR). The resistant check, ICC 4958, showed lower DSI values of root scanner, stereo microscope, and DSLR camera, indicating partial resistance. Test genotypes displayed a range of responses depending on the imaging technique.

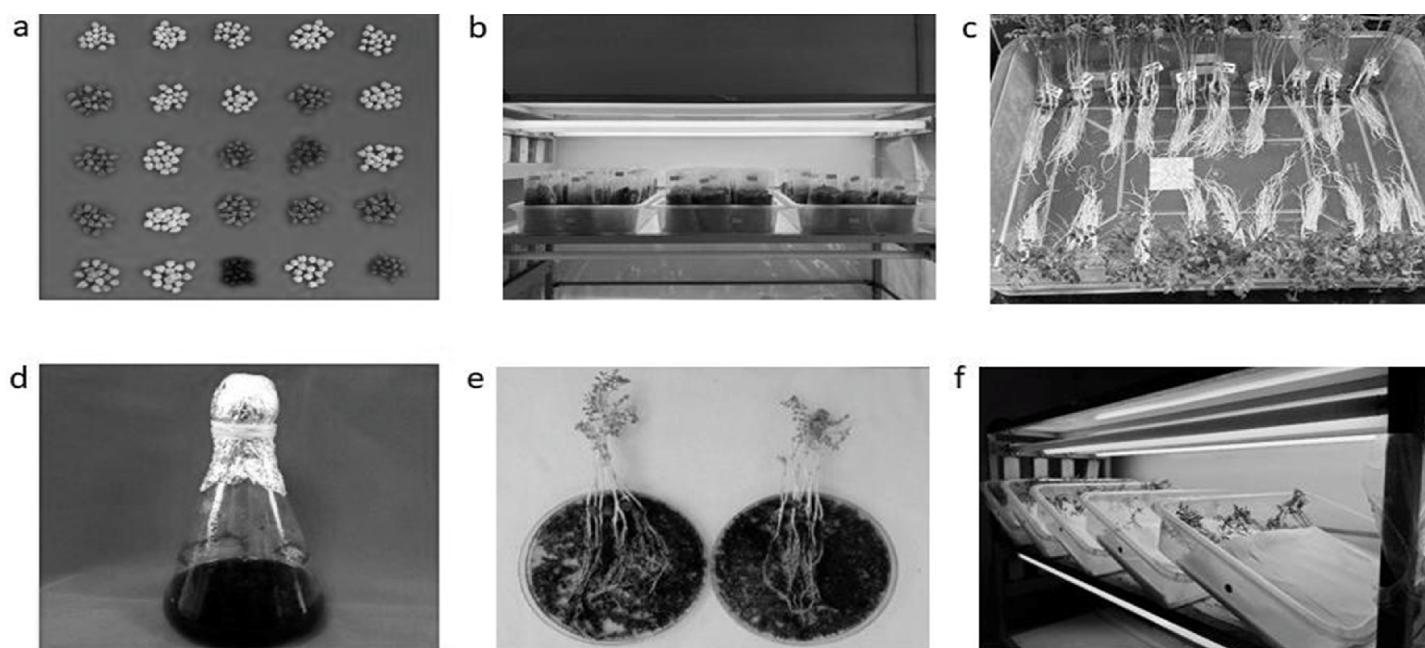


Fig. 1 : Images showing different Steps conducted for germination and inoculation of chickpea genotypes for scoring DRR data using blotting paper method.



Fig. 2 : Images of chickpea genotypes IC-83325 IC- 552241, IC-348528, IC-305467 and IC-95167 acquired from: (a) root scanner (b) stereomicroscope and (c) Growth of Chickpea (DSLR Camera Images).

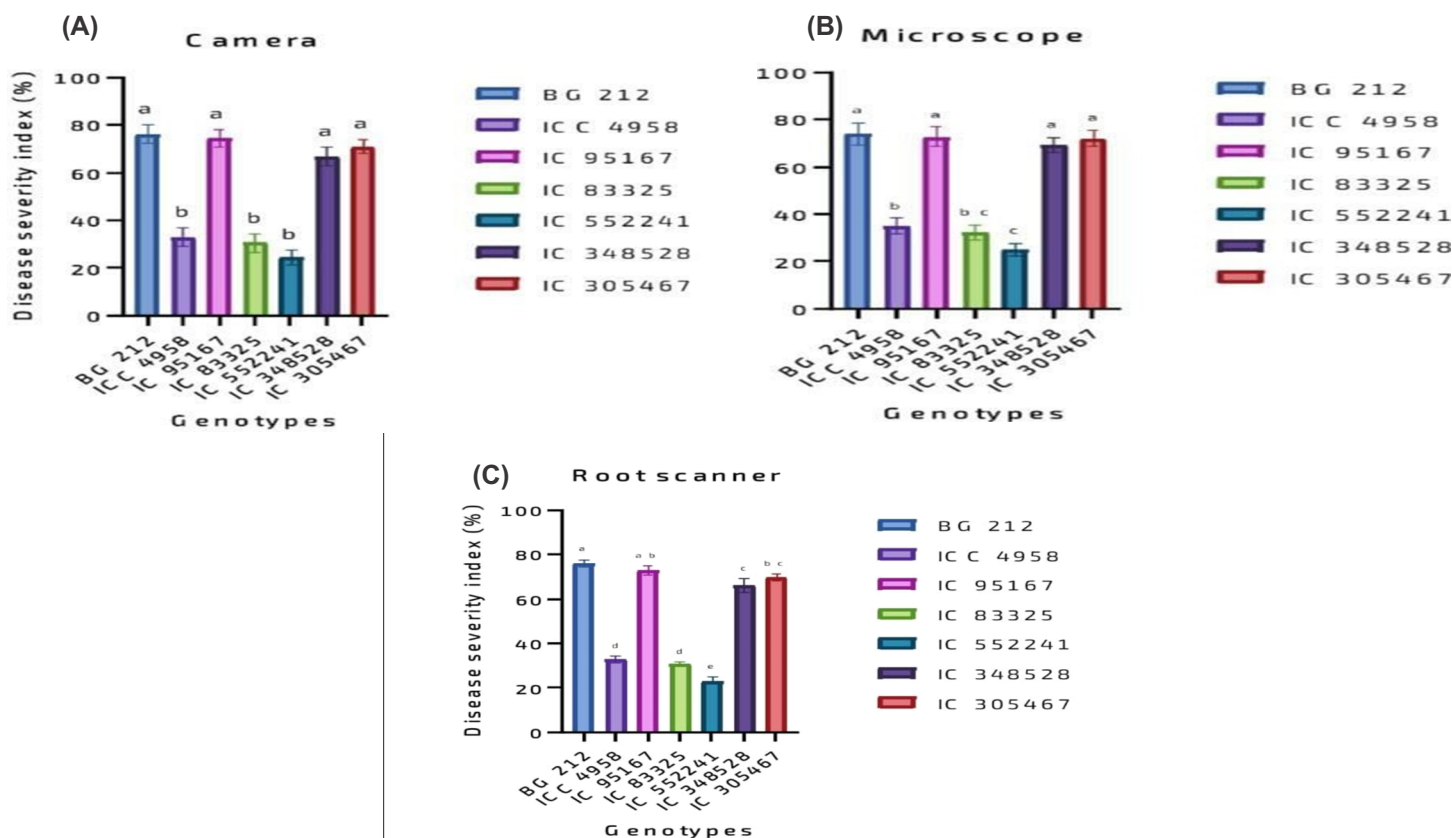


Fig. 1 : Bar graph of DSI (%) using **a)** DSLR camera **b)** stereo zoom microscope and **c)** root scanner image acquisition methods

Using the root scanner, DSI ranged from 28% to 82%. ICC 348528 exhibited the lowest DSI at 28% ('d' group), followed by ICC 552241 (32%, 'd' group) and ICC 83325 (38%, 'd' group), suggesting strong resistance. ICC 305467 showed an intermediate DSI of 62% ('b' group), while ICC 95167 (72%, 'a' group) and BG 212 (82%, 'a' group) were highly susceptible. ICC 4958 (78%, 'a' group) indicated moderate resistance.

The stereo zoom microscope revealed DSI ranging from 20% to 85%. ICC 348528 (20%, 'd' group), ICC 552241 (25%, 'c' group), and ICC 83325 (35%, 'c' group) displayed the lowest values, reinforcing their resistance. ICC 305467 (60%, 'b' group) and ICC 95167 (65%, 'b' group) showed moderate severity, while ICC 4958 (80%, 'a' group) and BG 212 (85%, 'a' group) had the highest DSI, with ICC 4958's value unexpectedly high for a resistant check.

The DSLR camera imaging yielded DSI values from 28% to 82%. ICC 348528 (28%, 'b' group), ICC

552241 (32%, 'b' group), and ICC 83325 (38%, 'b' group) again showed lower DSI, consistent with resistance. ICC 305467 (62%, 'a' group) and ICC 95167 (72%, 'a' group) had higher severity, while ICC 4958 (78%, 'a' group) and BG 212 (82%, 'a' group) were the most susceptible.

Statistical analysis across methods indicated significant differences ($p < 0.05$), with ICC 348528, ICC 552241, and ICC 83325 consistently showing the lowest DSI, aligning with resistance, while BG 212's high DSI validated its role as a susceptible check.

The differential disease severity indices (DSI %) observed across chickpea genotypes using root scanner, stereo zoom microscope, and DSLR camera imaging validate the blotting paper method as an effective tool for high-throughput screening of DRR resistance, consistent with previous research (Irulappan and Senthil-Kumar, 2021; Pandey *et al.*, 2021). The high DSI values for the susceptible check, BG 212 (82–85%), across all

methods confirm its vulnerability to *Macrophomina phaseolina*, aligning with its expected response under controlled conditions (Bampidis and Christodoulou, 2011). Conversely, the resistant check, ICC 4958, displayed moderate DSI values (78–80%), suggesting partial resistance, which may reflect the method's sensitivity to inoculum concentration (10^5 CFU/mL) or environmental factors like high humidity (70%) enhancing fungal growth (Marquez *et al.*, 2021).

The consistently low DSI values for ICC 348528 (20–28%), ICC 552241 (25–32%), and ICC 83325 (35–38%) across all imaging methods indicate robust resistance, likely due to enhanced root structural integrity or antifungal compounds (Gupta and Sharma, 2015). The root scanner effectively captured overall root damage (DSI 28–82%), while the stereo zoom microscope, with its higher resolution, detected the lowest DSI (20% for ICC 348528), possibly due to its ability to identify early microsclerotia formation. The DSLR camera corroborated these findings (DSI 28–82%), providing macroscopic validation of necrosis and discoloration, enhancing the reliability of the assessment.

The intermediate DSI of ICC 305467 (60–62%) and ICC 95167 (65–72%) suggests partial resistance or susceptibility, potentially influenced by genotype-specific interactions with the pathogen. The unexpectedly high DSI for ICC 4958 (78–80%) as a resistant check warrants further investigation, possibly indicating an atypical response to the controlled environment or a need for recalibration of resistance criteria. This discrepancy highlights the importance of including both susceptible (BG 212) and resistant (ICC 4958) checks to establish a baseline, though the absence of a second susceptible genotype (e.g., JG 62) limits comparative validation.

The integration of multiple imaging techniques provided complementary insights, with the root scanner offering a broad morphological overview, the microscope detecting fine pathogen details, and the DSLR camera confirming visual symptoms. However, the high DSI in ICC 4958 suggests potential limitations in inoculum

standardization or the need for field validation under natural drought stress, where DRR is most severe (Gaur *et al.*, 2010). ICC 348528, ICC 552241, and ICC 83325 emerge as promising candidates for breeding programs. The blotting paper method proved effective for high-throughput screening of chickpea genotypes for DRR resistance, consistent with previous studies. The method's advantages include rapid assessment (7 days), low cost, and controlled inoculum application, making it suitable for large-scale screening. Imaging techniques enhanced the precision of disease assessment. The root scanner provided detailed root morphology data, while DSLR and stereo microscope images enabled macroscopic and microscopic confirmation of symptoms and fungal structures, respectively. These tools facilitated quantitative analysis of necrosis and microsclerotia, improving upon traditional visual scoring methods.

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Significant Biomedical Applications of Hydrogels: An Overview

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Abstract

Hydrogels have gained significant attention due to their remarkable properties, including high water content, softness, flexibility, and excellent biocompatibility. These materials are formed by physically or chemically cross-linking natural or synthetic hydrophilic polymers, resulting in a three-dimensional network capable of retaining large amounts of water. Their close resemblance to living tissue makes them highly suitable for a wide range of biomedical applications. Today, hydrogels are widely used in the production of contact lenses, tissue engineering scaffolds, drug delivery systems, wound dressings and hygiene products. This study offers a comprehensive analysis of their key characteristics and diverse biomedical uses. From Otto Wichterle's groundbreaking work to the latest hydrogel-based innovations in commercial products, it provides readers with a thorough introduction to the field and a forward-looking perspective on future developments.

Keywords: *Hydrogels, drug delivery, contact lenses, tissue engineering, wound dressings, hygiene products, biomedical materials.*

Introduction

Hydrogels are three-dimensional networks formed by physical or chemical crosslinking of hydrophilic polymeric macromolecules, enabling them to absorb and retain large amounts of water while restricting its mobility through intermolecular interactions (Darge *et al.*, 2019; Di *et al.*, 2019). Over the past two decades, hydrogels have attracted considerable attention in chemical, biological, and pharmaceutical sciences due to their intrinsic features, including biocompatibility, biodegradability, tunable hydrophilicity (Ferreira *et al.*, 2018), and high absorption capacity. These properties make them valuable in enhancing the temporal and spatial delivery of macromolecular drugs, small molecules, and growth factors, thereby broadening their clinical utility across biomedical applications.

Hydrogels derived from natural or synthetic hydrophilic polymers often display improved

swellability and absorption due to the incorporation of functional groups such as amide, amino, carboxyl, and hydroxyl moieties within the polymer chain. Despite these advantages, hydrogel-based drug delivery systems still face challenges related to stability and performance. Natural polymers, for example, often suffer from limited thermal stability, low absorption capacity, and poor gel strength (Ferreira *et al.*, 2018). To address these shortcomings, synthetic polymers are increasingly employed as biomaterials for hydrogel preparation, offering more predictable physicochemical and mechanical properties (Abourehab *et al.*, 2022; Pramanik *et al.*, 2021).

The mechanical and functional performance of hydrogels can be further tailored by incorporating stimuli-responsive moieties. Such chemical modifications enhance structural resilience and enable "smart" behavior, allowing hydrogels to respond dynamically to environmental cues (Ferreira *et al.*, 2018; Chai *et al.*, 2017). Stimuli-responsive hydrogels, also termed intelligent hydrogels, are characterized by their ability to undergo reversible changes in swelling, volume, or sol-gel transitions in response to triggers such

as pH, temperature, light, electric fields, or ionic strength (Liu *et al.*, 2015; Guragain *et al.*, 2015).

Among these, thermoresponsive hydrogels have been particularly well studied. These systems can mimic aspects of living tissues and act as efficient carriers for bioactive molecules. Their unique behavior arises from the delicate balance between hydrophilic and hydrophobic components of the polymer. Temperature fluctuations alter polymer–water interactions, inducing solubility changes and reversible sol–gel transitions (Bajpai *et al.*, 2008; Ward and Georgiou 2011).

Depending on their design, some hydrogels shrink below and dissolve above the upper critical solution temperature (UCST), while others undergo a sol–gel transition above the lower critical solution temperature (LCST). These features are especially useful for applications requiring delayed drug release, extended skin retention, or reduced systemic side effects.

Thermoresponsive hydrogels thus represent a promising platform for advanced biomedical systems, as their sensitivity to environmental conditions closely parallels biomolecular processes in *in vivo* microenvironments. While numerous studies have investigated their synthesis, properties, and applications (Ferreira *et al.*, 2018; Di *et al.*, 2019), this review emphasizes an often-overlooked perspective: the patent literature. By focusing on patents, this study highlights the translational and commercial potential of hydrogel technologies in key biomedical fields, including contact lenses, tissue engineering, wound dressings, drug delivery and hygiene products.

Biomedical Applications of Hydrogels

Contact Lenses

Contact lenses are broadly categorized as 'hard' or 'soft' depending on their elasticity. Although hard lenses are more durable, they are less popular among users due to their limited comfort and longer adaptation periods. Hard lenses are typically composed of hydrophobic polymers such as poly(methyl methacrylate) (PMMA) or poly(hexa-fluoroisopropyl methacrylate)

(HFIM), whereas soft lenses are manufactured from hydrogels (Loyd *et al.*, 2002). Several fabrication techniques are available for soft lenses, including spin-casting, mold-casting, and lathe-cutting. In both spin- and mold-casting, a liquid monomer mixture is dispensed into a concave mold, where polymerization occurs under controlled thermal conditions, followed by the removal of unreacted monomers. Spin-casting involves rotation of the mold, allowing uniform distribution of the monomer, while mold-casting uses paired convex–concave molds to shape both surfaces of the lens (Hamilton and McFarlane, 2006). Innovative mold designs for silicone hydrogel (SiHy) lenses, such as those described in US Patent 6,861,123 B2 by Johnson & Johnson Vision Care Inc., have advanced precision and reproducibility in lens production (Maldonado and Efron, 2003).

An alternative manufacturing route, lathe-cutting, involves shaping lenses from solid polymer 'buttons' produced via bulk polymerization. These polymer buttons are formed in water tanks using free-radical initiators, which generate high-molecular-weight chains through thermal decomposition. Although oxygen-mediated degradation can occur on exposed surfaces, this is eliminated during the lathing process, as lenses are cut from the interior of the buttons (Maldonado and Efron, 2003). This approach remains relevant for custom lens production, despite being less scalable compared to mold-based techniques.

Considerable research has focused on developing next-generation lenses with improved mechanical and optical performance. For example, Kunzer and colleagues (Kunzler and Friends, 1991) demonstrated that incorporating specific hydrophobic monomers as copolymers with hydrophilic components such as HEMA or N-vinyl-2-pyrrolidone (NVP) enhances oxygen permeability and mechanical strength. Similarly, Lai and Quinn (Lai and Quinn, 1999) proposed the use of optically clear silicone thermoplastic hydrogels, which combine physical robustness with superior oxygen diffusion. However, a persistent drawback of traditional soft hydrogels



is their limited oxygen permeability, which can lead to corneal hypoxia. SiHy lenses have addressed this limitation by incorporating silicon-based monomers into crosslinked hydrogel networks, enabling higher oxygen transmission while maintaining hydration and comfort (Nichols, 2013).

Despite these advantages, SiHy lenses exhibit increased protein deposition compared to conventional hydrogels, which can compromise lens clarity and biocompatibility. To mitigate this, strategies such as surface modification and incorporation of anti-fouling additives have been investigated. For instance, European Patent EP 2 365 360 A2 describes the inclusion of compounds such as butylated hydroxytoluene (BHT) or hydroxyamines in the polymerization mixture to reduce protein uptake (Pinsley *et al.*, 2011). In addition, US Patent 3,808,178 details silicone-containing monomers that enhance lens performance, while plasma surface treatments have been employed to increase wettability and tear film stability (Gaylord, 1974; French, 2005; Steffen and Schnider, 2004). Wettability, commonly assessed via contact angle measurements, is a critical determinant of lens performance, with lower angles indicating better tear film spreading (French, 2005). Recently, Bauman *et al.* (Bauman *et al.*, 2012) reported the fabrication of SiHy lenses with nano-textured surfaces that mimic corneal topography, improving comfort and tear film stability.

Further innovations continue to emerge, such as the 'Gentle 59' lens developed by Vista Optics Ltd., composed of acrylic acid-co-acrylamide hydrogels with improved tensile strength, hydration, and comfort (Vista Optics, 2013). Beyond their role in vision correction, hydrogel lenses are being explored as platforms for ocular drug delivery. Conventional hydrogel lenses, however, suffer from limited drug-loading capacity and rapid burst release. To overcome these limitations, approaches such as modifying the hydrophilic/hydrophobic balance of copolymers, incorporating drug-loaded colloidal carriers, embedding ligand-functionalized hydrogels, or designing multilayer structures

have been reported (Hu *et al.*, 2011). For example, Venkatesh *et al.* (Venkatesh *et al.*, 2007) demonstrated the use of biomimetic hydrogels capable of sustained release of H1-antihistamines over five days. Similarly, Xu *et al.* (Xu *et al.*, 2010) incorporated β -cyclodextrin into PHEMA-based hydrogels to enhance drug loading and tensile strength. Using puerarin as a model drug, they observed prolonged ocular retention *in vivo* compared to standard hydrogel lenses and eye drops.

The continued development of hydrogel-based contact lenses reflects the industry's efforts to achieve an ideal balance of oxygen permeability, comfort, wettability, and biocompatibility, while also enabling therapeutic delivery. Despite significant advances, no single lens material has yet achieved all desirable properties, leaving considerable opportunities for innovation in the design of next-generation ophthalmic devices.

Tissue Engineering

Millions of patients worldwide suffer annually from the loss or failure of organs and tissues due to accidents or disease, with an estimated global economic burden reaching several trillions of dollars each year. While organ and tissue transplantation remains a well-established therapy, its widespread application is hindered by severe donor shortages (Lee and Mooney, 2003). This limitation has driven growing interest in tissue engineering, a field first formally defined in 1988 as the application of engineering and life sciences principles to elucidate the structure-function relationships of mammalian tissues and to develop biological substitutes capable of repairing or regenerating tissue and organ function (Chapekar, 2000). The overarching aim of tissue engineering is to improve or replace damaged tissues or organs through engineered materials and synthetic strategies.

Among the various materials investigated, hydrogels have emerged as particularly promising candidates in tissue engineering. Their unique characteristics allow them to serve as space-filling agents, vehicles for drug and bioactive molecule delivery, or three-dimensional



scaffolds that provide physical support and biochemical cues to guide cell growth and tissue formation. As space-filling agents, hydrogels can act as bulking materials, anti-adhesion barriers, or biological “glues.” In scaffold applications, they have been utilized for localized drug release to promote angiogenesis, as encapsulating matrices for secretory cells, and in tissue engineering strategies targeting cartilage, bone, and smooth muscle regeneration (Drury and Mooney, 2003).

A critical property for the successful application of hydrogels in tissue engineering is biocompatibility, defined as the ability of a material to interact with host tissues without eliciting adverse reactions (Chen *et al.*, 2000). Hydrogels can be derived from both synthetic and natural polymers. Synthetic examples include poly(ethylene oxide), poly(vinyl alcohol), poly(acrylic acid), poly(propylene fumarate-co-ethylene glycol), and polypeptides, while natural counterparts include agarose, alginate, chitosan, collagen, fibrin, gelatin, and hyaluronic acid (Jabbari *et al.*, 2006; Hunt *et al.*, 2014). Their wide versatility enables the design of systems with tailored degradation rates, porosity, and mechanical strength.

Several innovative approaches to hydrogel-based scaffolds have been reported in the literature and patents. For example, European Patent EP 1 664 168 B1 describes a hydrogel-based composition for manufacturing porous scaffolds using a biodegradable unsaturated self-cross-linkable polymer, hydrogel microparticles (1–1000 μm), and a free-radical initiator. The microparticles—often cross-linked collagen or gelatin—can encapsulate bioactive agents and swell extensively without losing structural integrity. Following polymerization, the scaffold can be applied directly to treat skeletal defects without leaching out porogens (Jabbari *et al.*, 2006). Similarly, Harris *et al.*, 2011 developed scaffolds combining microporous and nanoporous features, utilizing self-assembling peptide-based hydrogels that are biodegradable, non-immunogenic, and supportive of cellular interaction. These materials facilitate tissue

ingrowth and vascularization, while also functioning as slow-release drug delivery platforms.

Hydrogel scaffolds may also be engineered as hybrid materials, which often outperform single-component systems in supporting cell proliferation, migration, and differentiation. For instance, Kumar, 2013 disclosed biodegradable poly(vinyl alcohol)-based hydrogels complexed with phenylboronate polymers that enable controlled tissue growth. After culturing keratinocytes or fibroblasts on the scaffold for 5–20 days, the scaffold can be dissolved using saccharide solutions (e.g., fructose or mannitol), allowing for cell-sheet harvesting. Similarly, Blanchard *et al.*, 2002 reported keratin-based hydrogels—derived from patient hair or nails—that form cross-linked, hydrophilic scaffolds promoting epithelialization and nutrient delivery. Song *et al.*, 2013 further advanced the field by developing beta-glucan-based hydrogels through radiation fusion technology. These scaffolds, produced from beta-1,6-branched-beta-1,3-glucans, support stem cell adhesion, proliferation, and differentiation, while also stimulating collagen biosynthesis.

Despite these advances, significant challenges remain. A major hurdle in tissue engineering is the development of scaffolds that can support vascularization, a prerequisite for the long-term survival and integration of engineered tissues. Hydrogels present a promising solution by enabling localized delivery of angiogenic growth factors and endothelial cells (Lee and Mooney, 2001). However, many tissues such as bone, muscle, and blood vessels require excellent mechanical performance, a property most conventional hydrogels lack. Addressing this limitation will require the development of next-generation hydrogel systems with enhanced structural integrity, tunable mechanics, and bioactive functionalities to more effectively mimic native tissue environments and promote functional regeneration.

Wound Dressings

Wound is defined as a defect or break in the skin



resulting from trauma or medical or physiological conditions. Wounds can be classified according to the number of skin layers and the area affected: superficial wounds involve only the epidermis, partial-thickness wounds affect both the epidermis and dermal layers, while full-thickness wounds extend into the subcutaneous fat and deeper tissues (Eccleston, 2007). Wounds are further subdivided into acute or chronic categories. Chronic wounds, in particular, require intensive nursing care and represent a significant economic burden on national healthcare systems.

The design of effective wound dressings depends on a thorough understanding of the healing process, patient-specific conditions, and the effects of different materials on wound repair (Eccleston, 2007; Medaghieie *et al.*, 2014). Healing may be impaired by factors such as desiccation, infection or abnormal bacterial presence, maceration, necrosis, trauma, edema, or pressure (Turner, 1979). An ideal wound management product should be able to absorb excess exudate and toxins, maintain an appropriate level of moisture at the wound site, protect the wound from external infection, prevent overheating, allow adequate gas exchange, be completely sterile, and permit painless removal without causing further trauma (Jones *et al.*, 2006).

In recent years, the wound dressing industry has emphasized not only clinical performance but also patient comfort, conformability, reduced frequency of dressing changes, cost-effectiveness, and extended shelf life (Jones *et al.*, 2006). Choosing the appropriate dressing for a specific wound type is therefore fundamental for optimizing healing outcomes and improving patient quality of life (Beldon, 2010).

Currently available wound dressings are commonly classified into low-adherent dressings, semipermeable films, hydrocolloids, hydrogels, alginates, foam dressings, and antimicrobial dressings (Turner, 1979). Although plain gauze remains one of the most widely used options due to its low cost and accessibility, it has notable limitations. Gauzes impregnated with iodine, zinc oxide/zinc ions, or petrolatum demonstrate improved performance, with iodine providing

antimicrobial properties and zinc oxide supporting wound cleansing and re-epithelialization (Agren, 1990; Murphy and Evans, 2012). However, gauze removal may cause trauma by stripping away newly formed epidermis (Stashak, 2004).

In contrast, advanced dressings are specifically designed to maintain a moist environment, which accelerates healing while preventing exudates from spreading to surrounding healthy skin (Stashak, 2004). The importance of a moist wound environment in promoting healing was first demonstrated by Winter in 1962 and has since been extensively validated (Osti and Osti, 2004). Hydrogels and hydrocolloids are two key product types developed for moist wound healing. Hydrocolloids, typically composed of sodium carboxymethylcellulose, gelatin, pectin, elastomers, and adhesives, are able to absorb mild to moderate exudate. Both hydrocolloids and hydrogels promote autolytic debridement, a process that facilitates removal of necrotic tissue (Turner, 1979).

Hydrofiber® (ConvaTec) dressings represent another innovation in wound care, as they capture moisture through the formation of a swollen gel structure that conforms closely to the wound bed, effectively forming a seal. These dressings are typically fabricated as hydrophilic, non-woven flat sheets that transform into soft gel sheets upon absorbing wound exudate (Jones *et al.*, 2006).

Hydrogels, in particular, have become widely used as debriding agents, moist dressings, and components of wound care pastes. Unlike hydrocolloids, they do not require wound exudate to form a gel and are therefore especially suitable for dry wounds (Murphy and Evans, 2012). Their so-called moisture-donor effect promotes autolytic debridement by enhancing collagenase activity and increasing hydration in necrotic tissues (Stashak, 2004). In addition, hydrogels can absorb and retain contaminated exudates within their crosslinked polymer network, thereby isolating bacteria, necrotic debris, and odor molecules. Due to their high water content, hydrogels also permit vapor and oxygen transmission, making them particularly



effective for chronic wounds such as pressure ulcers.

Taken together, these advances highlight hydrogels as multifunctional wound management systems that not only accelerate healing but also provide a platform for controlled drug delivery and tissue regeneration, underscoring their broader biomedical importance.

Drug Delivery

Hydrogels possess a porous structure that enables efficient drug loading and controlled release, offering significant advantages for drug delivery applications such as sustained release, which helps maintain a high local concentration of the active pharmaceutical ingredient over extended periods (Hoare and Kohane, 2008). Drug release from hydrogels can occur through various mechanisms, including diffusion-controlled, swelling-controlled, chemically-controlled, and environmentally-responsive processes. In diffusion-controlled systems, the drug diffuses through the hydrogel mesh or water-filled pores and is typically delivered via reservoir or matrix devices. Reservoir systems consist of a drug-containing core surrounded by a hydrogel membrane, often shaped as capsules, cylinders, spheres, or slabs, with a higher drug concentration at the center to ensure a consistent release rate (Bierbrauer, 2005). In contrast, matrix systems feature the drug uniformly dispersed or dissolved throughout the hydrogel's three-dimensional structure, allowing gradual diffusion outward.

Drug release occurs through the macromolecular mesh or pores of the polymer matrix, and under this diffusion-controlled regime the initial release rate scales with the square root of time rather than remaining constant as in reservoir systems (Bierbrauer, 2005).

In swelling controlled devices, the drug is dispersed within a glassy polymer matrix. Upon contact with biofluid, the polymer swells and extends beyond its original boundary, facilitating drug diffusion via polymer chain relaxation (Bierbrauer, 2005). This mechanism—known as

Case II transport—yields constant, time-independent release kinetics and is often termed anomalous transport because it couples polymer swelling with molecular diffusion (Peppas and Lowman, 1999).

Hydrogels have been developed in a variety of forms depending on the intended route of drug administration. For peroral delivery, they have been prepared as spherical beads ranging from 1 μm to 1 mm in diameter (Lee and Kim, 1991; Ahmad, 2013), as discs with a typical diameter of 0.8 cm and thickness of 1 mm, and as nanoparticles within the 10–1000 nm size range (Hamid *et al.*, 2008). In rectal delivery, hydrogel suppositories have been designed with conventional adult dimensions of 32 mm in length, incorporating a central cavity of 7 mm and a wall thickness of 1.5 mm (Cole *et al.*, 1990). Vaginal administration systems include hydrogel-based tablets with dimensions of 2.3 cm in height, 1.3 cm in width, and 0.9 cm in thickness (Karasulu *et al.*, 2004), as well as torpedo-shaped pessaries measuring 30 mm in length and 10 mm in thickness. For ocular drug delivery, hydrogels have been employed in the form of contact lenses with a conventional diameter of approximately 12 mm (Hu *et al.*, 2011), hydrogel microparticles included in eye drops with sizes below 10 μm (Ludwig, 2005), suspensions and ointments (Rajasekaran *et al.*, 2010), and circular inserts with a 2 mm diameter and a total weight of about 1 mg (Hornof *et al.*, 2003). Transdermal delivery has also benefited from hydrogel systems, with examples including dressings of variable size and implants such as discs with a 14 mm diameter and 0.8 mm thickness or cylindrical devices measuring 3 mm in diameter and 3.5 cm in length (Schlegel *et al.*, 2001; Omidian and Park, 2012).

Hydrogel-based dosage forms can be tailored into a variety of shapes to suit different administration routes. Topical hydrogels provide an effective platform for delivering therapeutics that alleviate skin conditions. For example, Nho *et al.*, 2008 formulated a PVA/PVP hydrogel loaded with extracts of *Houttuynia cordata*, elm, celandine, and *Canavalia gladiata* to treat atopic dermatitis. They dissolved PVA and PVP directly in the



plant-extract solution, cast it into the desired form, and allowed it to set into a physical gel. A freeze-thaw cycle introduced physical cross-links, and subsequent gamma, UV, or electron-beam irradiation initiated chemical cross-linking while sterilizing the product. The finished hydrogel was supported on a hydrophilic non-woven fabric sheet and sealed under an air-permeable polyethylene film for easy handling and wear.

Hydrogels are also ideally suited to transdermal iontophoretic delivery. European Patent Application EP0524718A1 describes polyurethane hydrogel matrices used as monolithic drug reservoirs. These matrices conform closely to the skin, maintain ion conductivity, and enable precise, electrically driven transport of drugs across the barrier.

Hydrogel matrices can be loaded with drugs for controlled release. Transdermal iontophoresis is defined as the transport of ionic drugs across the skin under the influence of a weak electric current. The applied current facilitates the penetration of ionized molecules through the stratum corneum into the dermis, from which the active compound can diffuse into capillaries and enter systemic circulation. Alternatively, hydrogel formulations may serve as passive transdermal reservoirs. The hydrogels employed in such systems typically exhibit a high swelling ratio, good flexibility, mechanical strength, and optical transparency (Schlegel *et al.*, 2001).

Hydrogels are also promising carriers for ocular drug delivery, beyond their established use in contact lenses. For example, US Patent 8,409,606 B2 describes a system for the sustained release of therapeutic agents via punctal plugs. In this approach, very soft, biodegradable, covalently crosslinked hydrogels with high swelling capacity are designed to remain in situ (within the punctum or lacrimal canal) while maintaining patient comfort. Depending on therapeutic needs, plugs can be designed as temporary (collagen-based) or permanent (silicone-based) devices (Nho *et al.*, 2008).

Ocular Therapeutics™ has developed ophthalmic

drug delivery systems and medical devices based on poly(ethylene glycol) hydrogels. A notable example is the dexamethasone punctal plug, designed to provide controlled corticosteroid release for the treatment of post-operative inflammation and pain. Currently in Phase 3 clinical trials, the plug delivers the drug over a four-week period before being naturally eliminated via the nasolacrimal system (Soonkap, 1993).

Ideally, a drug delivery platform should be synchronized with the patient's physiological status, releasing the drug only when required. Unnecessary release may not only reduce efficacy but also increase the risk of side effects. Stimuli-responsive hydrogels address this challenge by altering their swelling behavior, permeability, or mechanical properties in response to internal or external triggers (Lee and Kim, 1991).

For instance, Bae *et al.*, 1993 proposed a delivery device in which a porous, “sponge-like” hydrogel matrix—confined within a permeable walled structure—releases its drug cargo upon deswelling in response to physical or chemical stimuli. Such systems can be based on temperature-sensitive copolymers (e.g., N-isopropylacrylamide derivatives) or pH-sensitive crosslinked weak polyelectrolytes, both of which are widely studied in controlled drug delivery.

Hydrogels can also be engineered to provide self-regulated or pulsatile drug release. A notable example is described in US Patent 5,514,380, which reports on biodegradable and non-toxic multiblock hydrogel copolymers synthesized from hydrophilic soft segments and biodegradable hard blocks. The hydrophilic domains were based on polyethylene oxide (PEO) or PEO/polypropylene oxide (PPO) copolymers with molecular weights between 600 and 30,000 Da, while the biodegradable domains were composed of polylactide (PLA), polyglycolide (PGA), or their copolymers. These copolymers undergo degradation through hydrolysis of ester and amide bonds under physiological conditions, allowing controlled drug release (Song *et al.*, 1996). Another patented approach, described in US Patent 8,383,153 B2, utilizes

poly(amidoamine)-based hydrogels that are sensitive to both temperature and pH. Their molecular design reduces the risk of an initial burst release and ensures sustained delivery. These hydrogels can be synthesized in a single step via coupling between the secondary amine groups of a diamine compound, such as piperazine, and the vinyl groups of bisacrylamides including N,N'-methylenebisacrylamide or N,N'-ethylenebisacrylamide (Lee *et al.*, 2013).

Further innovation is seen in US Patent 7,066,904 B2, which describes a combined hydrogel-catheter system designed to immobilize significant amounts of drug and release it in response to external stimuli. The hydrogel component, based on pH-sensitive polymers such as (hydroxyethyl)methacrylate-co-methacrylic acid, undergoes volume phase transitions at specific pH values. Release can be triggered by altering the microenvironment using salt solutions such as sodium phosphate or sodium bicarbonate, which shift the pH within the ranges of 7.5–8.4 or 6.4–7.3 to induce swelling or contraction of the hydrogel matrix (Rosenthal *et al.*, 2006).

Several hydrogel-based systems have successfully reached the pharmaceutical market. One of the earliest examples is Cervidil®, a vaginal insert launched in 1995 for cervical ripening. Each device contains 10 mg of dinoprostone (prostaglandin E₂, PGE₂) within 271 mg of a crosslinked polyethylene oxide/urethane hydrogel, releasing the drug at a rate of approximately 0.3 mg per hour over 12 hours, triggered by hydrogel swelling in the vaginal environment (Cervidil, 2013). Building on this platform, Controlled Therapeutics Scotland Ltd. developed a misoprostol vaginal insert (MVI) with similar design, intended to induce labor by releasing misoprostol, a cytoprotective agent with uterotonic activity (Controlled Therapeutics, 2013). The same company is also developing a hydrogel-based buccal patch, Pilobuc™, designed for the controlled release of pilocarpine in the treatment of Sjögren's syndrome, an autoimmune disorder

characterized by impaired tear and saliva secretion (Controlled Therapeutics, 2013).

Another marketed hydrogel system is Supprelin® LA, a subcutaneous implant developed by Endo Pharmaceuticals for the treatment of central precocious puberty (CPP). This non-biodegradable hydrogel device contains 50 mg of histrelin acetate embedded in a polymer matrix composed of 2-hydroxyethyl methacrylate, 2-hydroxypropyl methacrylate, trimethylolpropane trimethacrylate, benzoin methyl ether, Perkadox-16, and Triton X-100. The implant delivers the drug continuously at an average rate of 65 µg per day for up to 12 months, after which it must be surgically removed (Endo Pharmaceuticals, 2014).

In addition to these marketed products, advanced hydrogel compositions have been investigated as gastric-retentive devices. Park and colleagues (Park, 1988; Chen *et al.*, 2000 (a and b); Park *et al.*, 2001) developed superporous hydrogels for long-term oral delivery, synthesized from (meth)acrylic acid or (meth)acrylamide combined with crosslinkers such as N,N'-methylenebisacrylamide and disintegrants like crosslinked carboxymethylcellulose or poly(vinylpyrrolidone). A gas-blowing technique was used to generate these materials, whereby polymerization and foaming occurred simultaneously with sodium carbonate or bicarbonate as the foaming agent. Timing was critical in this process, as polymerization had to initiate within minutes of foaming to trap gas bubbles effectively, yielding highly porous networks capable of prolonged gastric retention and sustained drug release.

Hydrogels represent one of the most versatile classes of biomaterials for drug delivery. Their highly hydrated, polymeric networks provide flexibility, transparency, and biocompatibility, while also enabling controlled drug loading and release. Depending on their design, hydrogels can act as passive reservoirs, stimuli-responsive matrices, or implantable depots, and have been successfully applied across multiple routes of drug administration. One of the most explored approaches is transdermal delivery, where

hydrogels are used either as passive reservoirs or in combination with physical enhancement techniques such as iontophoresis. In iontophoretic systems, a mild electric current facilitates the transport of ionized drugs across the stratum corneum into the dermis, from which the drug diffuses into systemic circulation. Hydrogel formulations provide the necessary swelling, mechanical strength, and flexibility for both passive and current-assisted transport (Soonkap, 1993).

Hydrogels have also found significant application in ocular drug delivery. Beyond their traditional role in contact lenses, soft, biodegradable hydrogels have been investigated for punctal plugs capable of controlled drug release. For instance, US Patent 8,409,606 B2 describes crosslinked hydrogels designed to remain in the lacrimal canal with minimal discomfort, offering either temporary (collagen-based) or permanent (silicone-based) drug delivery (Sawhney *et al.*, 2013). Clinical progress has also been made with poly(ethylene glycol)-based hydrogel devices from Ocular Therapeutix™, including a dexamethasone punctal plug currently in Phase 3 trials for the management of post-operative inflammation and pain. This device provides sustained corticosteroid release over a four-week period before being naturally cleared via the nasolacrimal system (Ocular Therapeutix, 2014).

The ability of hydrogels to respond to physiological stimuli makes them particularly attractive for “smart” drug delivery. Systems sensitive to pH, temperature, ionic strength, or glucose concentration can undergo reversible changes in swelling, permeability, and mechanical properties, thereby enabling on-demand release. Bae *et al.*, 1993, for example, proposed a porous, sponge-like hydrogel matrix confined within a permeable structure that deswells under external stimuli to modulate drug release. Such systems are often based on temperature-sensitive N-isopropylacrylamide copolymers or pH-responsive weak polyelectrolytes, both of which have been widely investigated for drug delivery applications.

Across different routes of administration,

hydrogels have been designed in various forms, including spherical beads, discs, nanoparticles, suppositories, vaginal tablets, pessaries, contact lenses, eye drops, ocular inserts, dressings, and implants (Lee and Kim, 1991; Karasulu *et al.*, 2004; Ludwig, 2005; Hamidi *et al.*, 2008; Rajasekaran *et al.*, 2010; Hu *et al.*, 2011; Ahmad, 2013). This diversity underscores the adaptability of hydrogel systems to different therapeutic needs and anatomical sites.

Hydrogels have also been at the centre of numerous patented innovations. US Patent 5,514,380 reports biodegradable multiblock copolymers with hydrophilic polyethylene oxide or polyethylene oxide/polypropylene oxide domains and biodegradable polylactide or polyglycolide blocks, enabling degradation via hydrolysis (Song *et al.*, 1966). US Patent 8,383,153 B2 discloses poly(amidoamine)-based hydrogels sensitive to both pH and temperature, designed to reduce burst release and provide sustained delivery (Lee *et al.*, 2013). Similarly, US Patent 7,066,904 B2 introduces a hydrogel-catheter combination, where pH-sensitive matrices such as (hydroxyethyl)methacrylate-co-methacrylic acid undergo volume transitions in response to specific pH changes, thereby regulating drug release (Lee *et al.*, 2013).

Several commercial products highlight the clinical impact of hydrogel-based systems. Cervidil® (launched in 1995) is a vaginal insert containing 10 mg of dinoprostone embedded in a crosslinked polyethylene oxide/urethane hydrogel, designed for cervical ripening. The device swells upon contact with vaginal fluid, releasing approximately 0.3 mg of drug per hour over 12 hours (Cervidil, 2013). A similar platform has been adapted for misoprostol vaginal inserts (MVI) developed by Controlled Therapeutics Scotland Ltd. for induction of labor (Controlled Therapeutics, 2013). The same company is also advancing Pilobuc™, a buccal hydrogel patch delivering pilocarpine for Sjögren's syndrome, an autoimmune disorder characterized by reduced tear and saliva production (Controlled Therapeutics, 2013). Another marketed product, Supprelin® LA (Endo Pharmaceuticals), is a



subcutaneous hydrogel implant for the treatment of central precocious puberty. It contains 50 mg of histrelin acetate within a poly(2-hydroxyethyl methacrylate)-based matrix, releasing the drug at an average rate of 65 μg per day over 12 months. As the implant is non-biodegradable, it requires surgical removal after its therapeutic period (Endo Pharmaceuticals, 2014).

Beyond marketed therapies, advanced oral delivery systems have been explored. Park and colleagues (Park, 1988; Chen *et al.*, 2000; Park *et al.*, 2001) developed super porous hydrogels capable of gastric retention for more than 24 hours, enabling prolonged drug release. These systems were synthesized from (meth)acrylic acid or (meth)acrylamide using a gas-blowing technique in which polymerization and foaming occur simultaneously, with sodium carbonate or bicarbonate acting as the foaming agent. The resulting super porous structure allowed drugs such as NSAIDs (Velasco *et al.*, 2011), as well as proteins like insulin, to be delivered with enhanced stability and bioavailability. Insulin-loaded poly(methacrylic acid-g-ethylene glycol) hydrogels crosslinked with tetraethylene glycol dimethacrylate, as described in US Patent WO1998043615 A1, demonstrated protection in the gastric environment via pH-dependent inter-chain hydrogen bonding. At intestinal pH, the complexes dissociate, swelling occurs, and insulin is released, with strong mucoadhesive properties further enhancing absorption (Lowman *et al.*, 1998; Morishita *et al.*, 2002; Bumsang and Peppas, 2003).

Looking forward, hydrogel-based delivery systems are expected to play an increasingly central role in advanced therapeutics. By fine-tuning their hydrophilicity, crosslinking density, and structural characteristics, hydrogels can be engineered to achieve precise release profiles, targeting specific sites in the body at clinically relevant rates. This tunability is particularly advantageous for the delivery of sensitive biomolecules, such as therapeutic proteins and peptides, which remain difficult to administer effectively with conventional formulations. As research progresses, hydrogel platforms are

anticipated to make major contributions to the treatment of chronic and complex conditions, including diabetes and cancer, where localized, sustained, and responsive drug release can offer significant therapeutic benefits.

Superabsorbent Polymers and Hygiene Applications

Superabsorbent polymers (SAPs) were introduced into agriculture and hygiene industries more than three decades ago and have since been adopted across a wide range of applications due to their remarkable water retention capacity (Omidian *et al.*, 2005). The first commercial SAPs were launched in Japan in 1978 for feminine hygiene products and were based on crosslinked starch-g-polyacrylate (Masuda, 1994). By the early 1980s, SAPs entered the diaper market, driving significant improvements in performance. Their incorporation allowed diapers to become thinner, lighter, and more comfortable while achieving superior fluid retention and reducing leakage rates to below 2%. For example, Unicharm introduced SAPs into diapers in 1982, and this innovation rapidly spread to sanitary napkins and other hygiene products (Sannino *et al.*, 2009).

In the late 1990s, super porous hydrogels (SPHs) were developed as a distinct class of absorbent materials. Like SAPs, they consist of covalently crosslinked hydrophilic polymers, but they differ in their exceptionally fast, size-independent swelling kinetics. The first generation of SPHs was based on monomers such as acrylamide, acrylic acid salts, and sulfopropyl acrylate. Later "hybrid SPHs" incorporated natural or synthetic water-soluble polymers as hybrid agents, producing interpenetrating polymer networks with enhanced strength and elasticity even in the swollen state (Omidian *et al.*, 2005). These developments addressed limitations of earlier SAPs and expanded their potential uses.

Acrylate-based SAPs remain the dominant materials in disposable hygiene products. They efficiently absorb and retain large volumes of fluid while keeping skin dry, thereby reducing the risk of irritation and infection. Today, SAP-containing diapers are widely used worldwide,



both in domestic and clinical settings (Sannino *et al.*, 2009). However, their ecological impact is significant. For instance, a child up to 30 months of age may use around six diapers per day, generating over 1,000 m³ of waste per year (Sannino *et al.*, 2009). This has driven research into biodegradable absorbent systems. Promising alternatives include cellulose-based hydrogels, such as those composed of sodium carboxymethylcellulose (NaCMC) and hydroxyethyl cellulose (HEC), crosslinked with divinyl sulfone (DVS). These hydrogels display SAP-like swelling and water retention properties, aided by microporous structures that enhance absorption kinetics via capillary effects (Sannino *et al.*, 2009).

The importance of SAP technology is reflected in extensive patent activity. US Patent 32,649 described one of the first hydrogel-forming polymeric compositions for hygiene applications, prepared by polymerizing carboxylic or sulfonic acid monomers with crosslinking agents (Brandt *et al.*, 1988). Later patents, such as US 5,009,653, focused on sanitary napkins incorporating hydrogel-based absorbent cores, while US 5,147,343 combined fibrous matrices with SAPs to achieve “absorbency under load” (AUL), a key measure of fluid uptake under external pressure (Osborn, 1991; Kellenberger, 1992).

Globally, disposable diaper markets are dominated by Pampers (Procter & Gamble, ~35% share) and Huggies (Kimberly-Clark, ~22% share), with products sold in over 50 countries. Current industrial efforts focus on developing SAPs with higher AUL, lower residual monomer content, and improved safety (Zohuriaan-Mehr, 2008). Future advances are expected to include functionalized hydrogels containing antimicrobial enzymes or odor-control agents, as well as fully biodegradable hygiene products to address environmental concerns.

Conclusion

Hydrogels represent one of the most versatile classes of functional polymers. Their applications in contact lenses, hygiene products, and wound dressings are already well established, while their potential in drug delivery and tissue engineering

continues to expand. Although many innovative hydrogel-based systems have been designed and patented, relatively few have reached commercial success, in part due to production challenges and cost. Nevertheless, the field continues to evolve rapidly.

Future hydrogel technologies are expected to provide smarter, more responsive systems for therapeutic delivery and more sustainable, biodegradable solutions for large-scale applications such as hygiene. By combining advanced material design with environmentally conscious strategies, hydrogels are poised to play an even greater role in both healthcare and everyday life.

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India's First Gene-Edited Sheep: CRISPR Boosts Muscle by 30%

In a landmark achievement, scientists at Kashmir University, supported by ICAR funding, announced India's first gene-edited sheep. Using CRISPR-Cas9, they precisely disrupted the myostatin gene in a local Merino lamb—resulting in a remarkable 30% increase in muscle mass, aligning it with European Texel benchmarks. Crucially, no foreign DNA was introduced, creating a non-transgenic, gene-edited animal that sidesteps common regulatory and public acceptance concerns. This breakthrough illustrates the power of precision genome editing in livestock, enabling targeted enhancement of traits without textbooks methods like crossbreeding. Myostatin acts as a natural muscle limiter; by switching it off, researchers opened the door to more efficient meat production. Beyond boosting yield, the approach promises reduced feed-to-muscle conversion ratios and improved farm efficiency. India's gene-edited sheep also signals strategic self-reliance in biotechnology—it reduces dependence on imported genetics and positions domestic science at the forefront of agricultural innovation. As global demand for sustainable protein rises, India's capacity to tailor livestock breeds to local environments is increasingly valuable. As the pioneering lamb matures, its muscle gains will be closely monitored. If successful, this could lead to a national program rolling out gene-edited livestock for farmers—ushering in a new era for India's animal biotechnology industry.



Unveiling India's Ancient Mega-Snake: *Vasuki indicus*

Paleontologists working in central India uncovered the fossilized remains of a gargantuan snake named *Vasuki indicus*, which lived around 47 million years ago. Measuring over 15 meters (50 feet) in length and about 2 meters in width, this behemoth may outsize the famed Titanoboa by roughly 15 feet—making it potentially the largest snake ever discovered. Dubbed after the mythological serpent in Hindu lore, *V. indicus* inhabited prehistoric swamp environments and likely preyed on large vertebrates. The fossil find broadens understanding of Eocene ecosystems in the Indian subcontinent and suggests that early mammalian megafauna and large reptiles coexisted in warm, dense habitats. The enormity of *Vasuki* challenges assumptions about ancient reptile size limits. It hints that snake gigantism may have arisen independently in different paleoclimatic contexts. Indian researchers and international collaborators are now reconstructing its musculature and biomechanical capabilities using 3D modeling of vertebral elements. This mega-snake offers a glimpse into deep-time biodiversity and will likely spark renewed interest in India's paleontological potential.



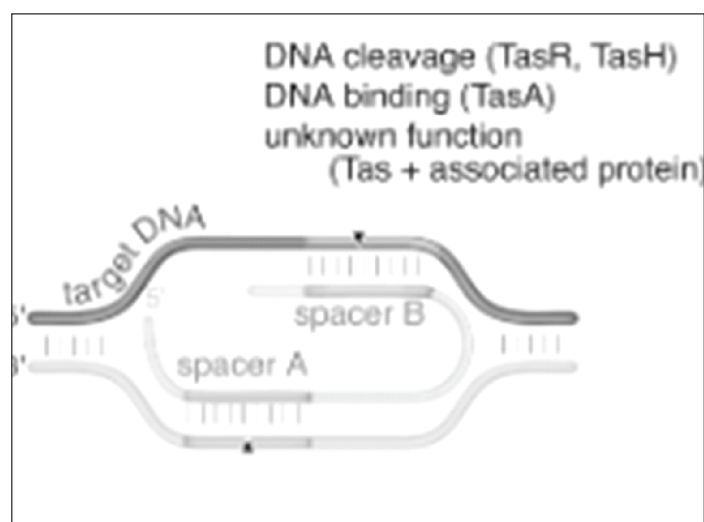
NIPGR's Climate-Smart Crop: Advika Chickpea

National Institute of Plant Genome Research (NIPGR) unveiled “Advika,” a new chickpea variety tailored for climate resilience and nutritional security. Built on the Indian Chickpea panArray, ADVIKA incorporates drought-tolerance and disease-resistance traits identified from sequencing thousands of global accessions. Field trials show robust performance in rainfed zones, offering up to 20% yield gains compared to existing varieties. Chickpea is a staple pulse crop in India—contributing significantly to protein intake. However, erratic rainfall and soil-borne diseases have limited productivity. Advika was developed via genomic-assisted breeding, focusing on a QTL hotspot linked to drought tolerance and resistance genes against Fusarium wilt. The result: a hardy, high-yielding cultivar ready for diverse agro-climates. In January 2025, the new variety was officially gazetted, paving the way for seed distribution through public and private channels. Speed-breeding techniques and high-throughput phenotyping facilities in collaboration with BIBCOT helped shorten the development timeline. Advika exemplifies India's emerging bioeconomy: leveraging “Make-in-India” genomics tools to secure national food systems. As it scales, Advika may reduce chickpea imports—India currently produces about 74% of global output yet still faces supply-demand gaps. Looking ahead, breeders are working on Next-Advika, combining nutrient density, pest tolerance, and broader stress adaptation. Advika not only enhances food security—it heralds a new generation of climate-smart crops powered by Indian innovation.



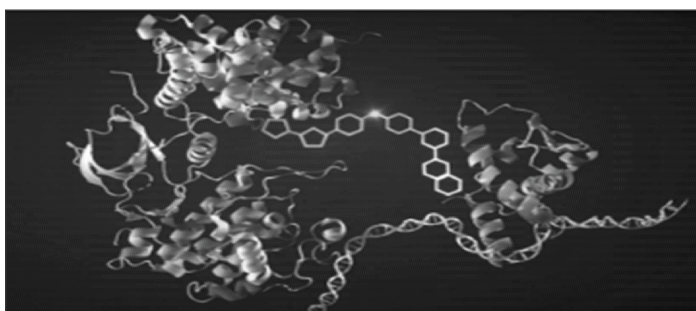
TIGR-Tas: Next-Gen Gene Editor Leaps Beyond CRISPR

In a blockbuster February 2025 announcement, researchers from MIT and the Broad Institute unveiled TIGR-Tas, a revolutionary RNA-guided gene editing system. Unlike CRISPR-Cas9—which relies on a single guide RNA and a PAM sequence—TIGR-Tas employs twin guide RNAs targeting opposite strands without PAM restrictions. This two-spacer design expands editable genome regions, drastically reducing off-target risks. TIGR-Tas is built on prokaryotic Tas proteins—nucleases that cleave with precise overhangs—and an RNA architecture reminiscent of snoRNAs. Its discovery through AI-powered protein mining highlights how computational biology accelerates biotechnology. With over 20,000 Tas variants identified in phage genomes, the system promises modularity and adaptability across organisms. Early plant cell tests demonstrate successful editing in PAM-limited regions and dual-strand precision. Although human applications are in early stages, its potential is vast: targeted treatments for genetic diseases, minimal off-target therapeutics, and improved crop trait engineering. TIGR-Tas showcases the next era of genome editing—more flexible, efficient, and safer—offering tools to address medical and agricultural challenges with unprecedented resolution.



esmGFP: AI-Invented Fluorescent Protein Decoding Biology Faster

In a pioneering blend of AI and synthetic biology, researchers unveiled esmGFP—an artificially designed fluorescent protein generated by ESM3, a deep-learning model trained on billions of natural protein sequences. Remarkably, esmGFP exhibits bright green fluorescence and structural stability, a design that would likely take nature 500 million years to evolve. Unlike traditional methods involving mutagenesis of existing proteins, AI predicted novel sequence–structure relationships, enabling the creation of a fully new fold not present in natural databases. Early lab tests confirm its folding behavior and fluorescence properties match or exceed those of GFP variants derived from jellyfish. esmGFP's implications for plant and animal biotechnology are profound. As a biological marker, it can be embedded into transgenes to monitor expression in crops or livestock cells—or track engineered bacterial consortia in greenhouse applications. More broadly, this experiment signals the dawn of AI-enabled protein design: synthetic enzymes, therapeutic antibodies, and biofabrication tools may soon be created *de novo*. Importantly, esmGFP is fully synthetic, with no natural origin or ecological history. Its deployment raises biosafety questions—especially regarding gene containment, unintended interactions, and transgenic regulation. As AI-designed biomolecules enter labs and fields, regulatory frameworks must adapt accordingly. Still, esmGFP's debut marks a paradigm shift. It expands biotechnology beyond the limits of natural evolution, enabling rapid, purpose-driven protein discovery—boosting the speed and scope of innovation in plant and animal biotech.



Hillchol: India's Oral Cholera Vaccine Completes Phase III Trials

In May 2025, Bharat Biotech achieved a major milestone with Hillchol—an indigenous, two-strain oral cholera vaccine designed to protect against Ogawa and Inaba serotypes. The successful Phase III trial involved thousands of participants across endemic regions, demonstrating safety and efficacy that met global benchmarks. Hillchol builds on Bharat Biotech's vaccine expertise, following revaxis efforts with rotavirus, typhoid, polio, and COVID-19 vaccines. It enhances gut immunity via oral administration—making it easy to deploy in rural and peri-urban settings. Cholera remains a persistent public health challenge in India, especially during monsoons and in resource-limited areas. The availability of a domestically manufactured oral vaccine promises to improve outbreak responsiveness and reduce reliance on global supply chains. Public Health experts see Hillchol as a key component of India's mission to eliminate cholera by 2030 under WHO guidance. Bharat Biotech is now preparing WHO prequalification and exploring integration into the Universal Immunization Programme. Hillchol exemplifies how biotechnology can directly improve public health and strengthen India's vaccine ecosystem. It also underscores the country's expanding capabilities in disease prevention and vaccine innovation—a cornerstone of global health resilience.

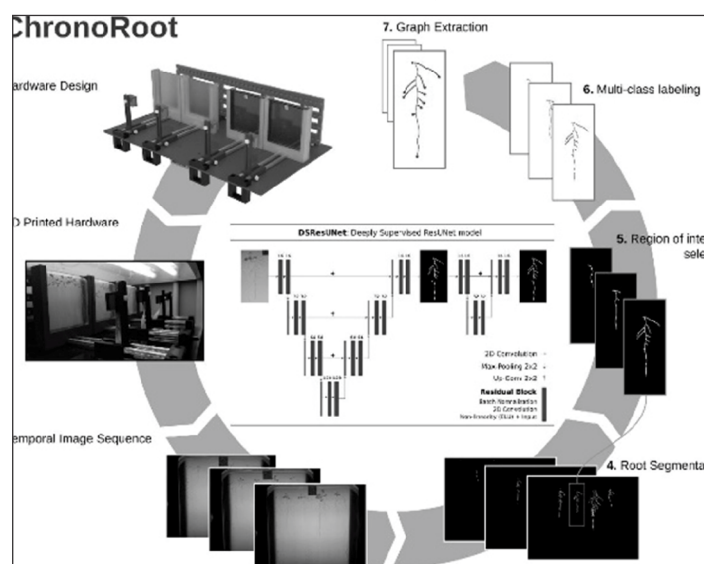


Smart Root Reader: ChronoRoot 2.0 Unveils Temporal Plant Behavior

A team of international researchers has launched ChronoRoot 2.0, an open-source platform that gives scientists unprecedented insight into plant root dynamics over time. By marrying low-cost hardware with AI-driven image analysis, the system tracks multiple organs—roots, cotyledons, hypocotyls—across developmental stages in *Arabidopsis thaliana* seedlings. Users can monitor circadian growth, gravitropic bending, and etiolated responses under various conditions. This tool transforms temporal phenotyping. Previously, sophisticated equipment limited phenotyping to lab-capable institutions; ChronoRoot democratizes access. Researchers can now identify traits like root architecture variability or stress responses using user-friendly software, potentially accelerating breeding of climate-ready crops. The open-source nature supports collaboration—labs worldwide can adapt the platform for diverse species or traits. In a pilot, scientists characterized light- and gravity-responsive growth patterns in transgenic lines, generating data at scale. The system's modularity means future versions could add nutrient stress measures or pathogen responses. ChronoRoot 2.0 isn't just another phenotyping tool—it's a roadmap for affordable, AI-enhanced plant experimentation. As climate challenges intensify, being able to track how roots, the unseen lifeline of plants, respond in real time could be critical to food security.

Himalayan Bat Mystery: *Myotis himalaicus* Joins India's Fauna

A joint Indo-European team, including the Zoological Survey of India, has confirmed the Himalayan long-tailed myotis (*Myotis himalaicus*) in Uttarakhand's Kedarnath Wildlife Sanctuary. First spotted during surveys in 2021 and identified in specimens dating back to 1998, this new species expands India's known bat diversity to 135 species. Distinguished by its elongated tail and distinct cranial features, *M. himalaicus* inhabits mid-elevation cedar and pine forests. Its discovery underscores the value of museum collections and global collaboration. Concurrently, the study also validated several other rare bat species in India. The find carries important conservation implications. Local guardians will now be trained to monitor bat populations, forming the backbone of community-based conservation. Bats play essential ecological roles—pollination, pest control, and fungus regulation—so documenting new species is a vital step toward forest health. This discovery highlights the significance of the Himalayan region as a biodiversity hotspot and a reservoir for cryptic wildlife.



NATIONAL AND INTERNATIONAL SEMINAR



GOVERNMENT-ORGANIZED NATIONAL SEMINARS

1. INFAH National Seminar 2025

- **Theme:** *Strengthening Animal Health and its Economics through Public-Private Partnership for Viksit Bharat*
- **Date:** April 29, 2025
- **Organized by:** Indian Federation of Animal Health Companies (INFAH)
- **Venue:** India Habitat Centre, New Delhi
- **Focus:** Enhancing animal health through collaborative efforts between public and private sectors.

2. Samajik Nyaya Charcha 2025

- **Date:** April 22, 2025
- **Organized by:** Ministry of Social Justice and Empowerment in partnership with The/Nudge Institute
- **Venue:** New Delhi
- **Focus:** Facilitating multi-stakeholder dialogue on advancing social equity and inclusive development in India.

MAJOR INTERNATIONAL ACADEMIC CONFERENCES IN 2025

1. IAU 2025 International Conference

- **Dates:** October 21–23, 2025
- **Location:** University of Rwanda, Kigali, Rwanda
- **Theme:** *Building Trust in Higher Education*
- **Organizer:** International Association of Universities (IAU)
- **Focus:** Exploring strategies to enhance trust in higher education institutions globally.

2. NAFSA 2025 Annual Conference & Expo

- **Dates:** May 2025
- **Location:** To be announced
- **Organizer:** NAFSA: Association of

International Educators

- **Focus:** International education, institutional partnerships, and global engagement strategies.

3. EAIE 2025 Annual Conference and Exhibition

- **Dates:** September 2025
- **Location:** Gothenburg, Sweden
- **Organizer:** European Association for International Education (EAIE)
- **Focus:** Internationalization of higher education, student mobility, and global partnerships.

4. AACSB International Conference and Annual Meeting (ICAM) 2025

- **Dates:** April 2025
- **Location:** Vienna, Austria
- **Organizer:** Association to Advance Collegiate Schools of Business (AACSB)
- **Focus:** Business education, accreditation standards, and innovation in management education.

5. SIGCSE Technical Symposium on Computer Science Education

- **Dates:** February 26 – March 1, 2025
- **Location:** Pittsburgh, Pennsylvania, USA
- **Organizer:** ACM Special Interest Group on Computer Science Education (SIGCSE)
- **Focus:** Advancements in computer science education, curriculum development, and pedagogy.

6. FPSAC 2025 – Formal Power Series and Algebraic Combinatorics

- **Dates:** July 21–25, 2025
- **Location:** Hokkaido University, Sapporo, Japan
- **Focus:** Algebraic combinatorics, formal power series, and their applications in mathematics and computer science.



7. ISAAC 2025 Congress

- **Dates:** July 2025
- **Location:** Nazarbayev University, Astana, Kazakhstan
- **Organizer:** International Society for Analysis, its Applications and Computation (ISAAC)
- **Focus:** Mathematical analysis and its applications across various scientific disciplines.

8. ATINER 2025 Conferences

- **Dates:** Various dates in July 2025
- **Location:** Athens, Greece
- **Organizer:** Athens Institute for Education and Research (ATINER)
- **Focus:** Multiple disciplines including environment, ecology, climate change, and higher education.

9. Forum on Education Abroad Annual Conference 2025

- **Dates:** March 19–21, 2025 (In-person); February 26–27, 2025 (Virtual)
- **Location:** Toronto, Canada
- **Focus:** Innovation and advocacy in education abroad programs and policies.

10. WEI International Academic Conference on Education, Humanities, and Social Sciences

- **Dates:** April 15–17, 2025
- **Location:** University of Miami, Miami, Florida, USA
- **Organizer:** West East Institute (WEI)
- **Focus:** Interdisciplinary research in education, humanities, and social sciences.

ONE DAY WORKSHOP

Strategic Thinking & Problem-Solving for Career Growth

Date: March 11, 2025

- **Organizer:** Vishwakarma University, Pune
- **Focus:** Enhancing critical thinking, problem-solving, and communication skills for career advancement.

- **Details:** Vishwakarma University Pune

Sustainable and Resilient Entrepreneurship

- **Date:** April 26, 2025
- **Organizer:** ABV-Indian Institute of Information Technology and Management, Gwalior
- **Focus:** Promoting sustainable entrepreneurship practices and resilience in business models.
- **Details:** IIITM

Advanced Functional Materials (AFM'25)

- **Date:** January 8, 2025
- **Organizer:** VIT-AP University
- **Focus:** Exploring advancements in functional materials and their applications.
- **Details:** VVIT-AP University

Design Thinking and The Business of AI

- **Date:** May 3, 2025
- **Organizer:** Dhirubhai Ambani Institute of Information and Communication Technology (DA-IICT), Gandhinagar
- **Focus:** Integrating design thinking principles with AI to drive business innovation.
- **Details:** DAIICT

Technical Writing Workshop

- **Date:** February 13, 2025
- **Organizer:** Nirma University, Ahmedabad
- **Focus:** Enhancing technical writing skills for academic and professional purposes.
- **Details:** Computer Science and Engineering

UGC-NET/SLET/JRF Preparation

- **Date:** March 22, 2025
- **Organizer:** Assam Don Bosco University
- **Focus:** Guidance and strategies for qualifying national eligibility tests.
- **Details:** Assam Don Bosco University

Intellectual Property Rights: European and Indian Perspectives

- **Date:** Date not specified
- **Organizer:** Vellore Institute of Technology



(VIT)

- **Focus:** Understanding IPR laws and practices in Europe and India.
- **Details:** Vellore Institute of Technology, Pune
- 48th Istanbul International Conference on Nursing, Medical, Environmental and Food Sciences, Date & Venue: December 15–17, 2025, Istanbul, Türkiye, Abstract Submission: August–September 2025, Registration: October 2025.
- Annual Meeting of the Indian Cancer Genome Atlas, Date & Venue: 14th to 16th November 2025, India, Abstract Submission: August 2025, Registration: September 2025
- Biotales 2025, Date & Venue: 26th to 27th July 2025, India, Abstract Submission: expected May 2025, Registration: June 2025
- Cardiac Cell Culture and Applications Molecular Techniques Workshop, Date & Venue: 17th to 18th July 2025, India, Abstract Submission and Registration: June 2025
- EMBO India Lecture Course on Neuroethology How the Brain Controls Natural Behaviours, Date & Venue: 7th to 11th December 2025, India, Abstract Submission: September 2025, Registration: October 2025
- Fifteenth Euro-Global Conference on Biotechnology and Bioengineering, Date & Venue: 18th to 20th September 2025, London, United Kingdom (hybrid), Abstract Submission: June 2025, Registration: August 2025
- Forty eighth All India Cell Biology Conference and Symposium, Date & Venue: 7th to 9th December 2025, India, Abstract Submission: September 2025, Registration: October 2025
- Genomics India Conference 2025, Date & Venue: 12th to 14th August 2025, India, Abstract Submission: June 2025, Registration: July 2025
- International Conference on Advances in Biotechnology Bioprocessing and Structural Biology, Date & Venue: 11th to 14th December 2025, Indian Institute of Technology Roorkee, Roorkee, India, Abstract Submission: August 2025, Registration: October 2025
- International Conference on Cell Science and Molecular Biology, Date & Venue: July 5, 2025, Dallas, Texas, United States, Abstract Submission: March 2025, Registration: May 2025.
- International Conference on Environment and Life Science, December 1, 2025, Gomel, Belarus.
- International Conference on Food Science Research, Technology and Innovation, December 4, 2025, Sydney, Australia.
- International Conference on Public Health Nutrition, December 4, 2025, Auckland, New Zealand.
- International Society for Computational Biology Basel Computational Biology Conference, Date & Venue: July 2025, MCH Congress Center Basel, Basel, Switzerland, Abstract Submission: March 2025, Registration: April 2025.
- ISMB/ECCB 2025 Conference, Date & Venue: July 20–24, 2025, Liverpool, United Kingdom, Abstract Submission: April 2025, Registration: May 2025.
- National Conference on Biotechnology and Biomedicines, Date & Venue: 19th July 2025, Delhi, India, Abstract Submission: April 2025, Registration: June 2025
- National Conference on Biotechnology and Biomedicines, Date & Venue: 23rd August 2025, Mumbai, India, Abstract Submission: May 2025, Registration: July 2025
- National Conference on Physical and Life Science, Date & Venue: 19th July 2025, Hyderabad, India, Abstract Submission: April 2025, Registration: May 2025
- Nipah and Zoonotic Spillovers Integrating Health Strategies for Prevention Conference, Date & Venue: 11th July 2025, India, Abstract Submission: April 2025, Registration: May 2025



- Society for Industrial Microbiology and Biotechnology Annual Meeting, Date & Venue: July 27–30, 2025, Hyatt Regency San Antonio Riverwalk, San Antonio, Texas, United States, Abstract Submission: April 2025, Registration: May 2025.
- Spanish Society of Biotechnology 19th Congress, Date & Venue: July 15–18, 2025, Universitat Autònoma de Barcelona, Barcelona, Spain, Abstract Submission: April 2025, Registration: likely May 2025.
- Symposium on One Health Approach to Antimicrobial Resistance, Date & Venue: 29th to 31st October 2025, Indian Institute of Science, Bangalore, India, Abstract Submission: August 2025, Registration: September 2025
- World Health Summit 2025, Date & Venue: October 12–15, 2025, Berlin, Germany, Abstract Submission: June 2025, Registration: August 2025.
- World Health Summit, Date & Venue: 12th to 15th October 2025, Berlin, Germany and online, Abstract Submission: June 2025, Registration: August 2025

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January 01, 2025

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Sengar, R.S. (2013). Estimation of population growth and extinction parameters from noisy data. *Ecol. Appl.*, **13** : 806–813.

Sirova, D., Adamec, L. and Verba, J. (2013). Enzymatic activities in tropes of four aquatic pieces 3 of the Carnivorous genus *Vlricularia*, *New Phytology* **159**(3) : 669-675.

Sengar, R.S., Sharma, A.K., Chaudhary, R. and Kureel, R.S. (2009). Biodiesel plant *Jatropha* for future. Proceedings of —5th World Congress of Cellular & Molecular Biology (WCCMB, 2012), November 02 - 06, School of Biotechnology, Devi Ahilya University Indore, India & World Society of Cellular & Molecular Biology, France. 142-143.

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