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# BIOTECH TODAY

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- To promote and undertake research and development and extension service in the field of agriculture and biological science.
- To explore new areas in agriculture, biological research, biotechnology crop cultivation technologies, development activity and logistics management.
- To propagate utilization on non-conventional and renewable sources in agriculture and biological research.
- To develop purely organic package for growing and cultivation of crops.
- To develop technology of in vivo propagation of important crops.
- To offer recognition and awards to professional groups and individual for attainment of excellence in the field of agriculture, biological science and Biotechnology.
- To interact with government agencies, scientific organization and NGOs to promote and protect interest of agriculture and biological science researcher.

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**REVIEW ARTICLE**

# Bioactive Compounds in Integrative Oncology and Cancer Therapeutics: A Comprehensive Review

**Shruti Morjaria[1], Neelesh Kapoor[2] and Rohit Kumar[3*]**

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## Abstract

Integrative medicine, a holistic approach that combines conventional medical practices with complementary and alternative therapies, has gained significant attention for its potential to provide comprehensive healthcare solutions. This review aims to elucidate the role of bioactive compounds in integrative medicine, focusing on their therapeutic effects, mechanisms of action and evidence-based applications in cancer treatment. The present review covers a range of bioactive compounds, including polyphenols, alkaloids, terpenoids, and more, which are found in natural sources such as plants, herbs and traditional remedies and highlight their role in cancer treatment as well as mitigating the adverse effect of cancer treatments. The integration of these compounds into conventional treatments highlights their potential to enhance patient outcomes and broaden the therapeutic landscape.

**Keywords:** bioactive compounds, integrative medicine, antineoplastic, evidence-based medicine.

## Introduction

Integrative oncology, an innovative and patient-centeric healthcare approach, has emerged as a promising strategy that integrates conventional medical practices with complementary and alternative therapies to comprehensively address a wide spectrum of health conditions (Grant *et al.*, 2019). Cancer treatment has been focused on majorly surgical oncology, chemotherapy, radiation therapy and immunotherapy (Siamof *et al.*, 2020), though there is a continuous effort undergoing to explore novel treatment options. In recent years, there has been a remarkable shift in the paradigm of healthcare and a more integrative approach is being used to stretch the treatment outcomes (O'Brien *et al.*, 2022). At the forefront of this evolving landscape are bioactive compounds, derived from natural sources, which have garnered increasing attention for their multifaceted therapeutic properties and potential to revolutionize patient care (Yuan *et al.*, 2022). Conventional medicine, rooted in evidence-based practices and advanced technologies, has undeniably made a significant contribution to diagnosing and treating a wide range of neoplasms. However, its focus on symptom management and disease-centric approaches often overlooks the interconnectedness of various systems and the broader context in which health is situated. Integrative medicine seeks to bridge the gap between these two paradigms, leveraging the strengths of both conventional and alternative approaches to provide patients with a comprehensive and personalized healthcare experience (Gannotta *et al.*, 2018).

A cornerstone of integrative medicine is the utilization of bioactive compounds derived

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from natural sources. These bioactive compounds are abundant in plants, herbs, fruits and other natural sources and have been utilized for centuries in traditional medicine systems across cultures. These compounds encompass a diverse array of molecules, including but not limited to polyphenols, alkaloids, terpenoids and flavonoids that exhibit a wide range of pharmacological activities (Biesalski *et al*., 2009).

This comprehensive review aims to explore the pivotal role of bioactive compounds and their role in integrative medicine. By delving into their potential applications, underlying molecular mechanisms and the mounting evidence supporting their efficacy, this review seeks to shed light on how these compounds contribute to the multifaceted landscape of integrative healthcare.

## Polyphenols in Integrative Medicine

Polyphenols, a group of naturally occurring compounds found abundantly in fruits, vegetables, herbs and other plant sources, have captured scientific attention due to their multifaceted biological effects. From their established role as antioxidants to their emerging roles in modulating tumor microenvironment, cell signalings and metabolic pathways, polyphenols offer a promising avenue for integrative cancer medicine. Oral supplementation with polyphenol-rich extract, specifically castalagin, alters gut microbial composition in mice, resulting in improved antitumor activity and enhanced anti-PD-1 immune response (Messaoudene *et al*., 2022). This compound promotes beneficial bacteria associated with effective immunotherapy responses and favorable changes in the tumor microenvironment, highlighting its potential in integrative medicine by augmenting cancer immunotherapy by modulating the gut microbiota and metabolic pathways. Another method discovered for resveratrol (RSV) mediated enhancement of immunotherapeutic response is the direct inhibition of enzymes involved in glyco-PD-L1 processing, leading to abnormal glycosylation and retention of PD-L1 within the endoplasmic reticulum (Verdura *et al*., 2020). RSV also demonstrates in-vivo reactive oxygen species (ROS) scavenging and when cancer cell lines were compared to normal ones RSV showed greater cytotoxicity through apoptosis induction and S-phase arrest with increased cell percentage and reduced G1/G0 phase cells upon treatment (Wu *et al*., 2019).

Polyphenols have demonstrated their potential in combating various types of cancer. In breast cancer, compounds like fucoxanthin and punicalagin inhibited metastasis (Malhao *et al*., 2021), suppressed angiogenesis and lymphangiogenesis (Ahmed *et al*., 2022; Wang *et al*., 2019) and induce apoptosis (Ahmed *et al*., 2022). Curcumin also stands out as a versatile polyphenol capable of inducing apoptosis in Colo-Rectal Cancer (CRC) (Calibasi-Kocal *et al*., 2019; Liu *et al*., 2023), lung (Jayakumar *et al*., 2017; Tang *et al*., 2021), breast (El-Far *et al*., 2022) and pancreatic cancer cells (Su *et al*., 2017). Lung cancer benefits from the anti-proliferative properties of anthocyanin (Alsharairi, 2022), while triterpenoids (Xu *et al*., 2018) and saponin (Teng *et al*., 2019; Wang *et al*., 2020) show promise as anticancer agents. Similarly, in pancreatic cancer, genistein and garcinol exhibit significant anticancer activity (Saadat *et al*., 2018). Limonoids exhibit anti-proliferative effects in pancreatic cancer (Chidambara Murthy *et al*., 2021), while carotenoids (Bakshi *et al*., 2022), -sitosterol (Gu *et al*., 2023) and polyphenol saponin (Xia *et al*., 2022) show potential in colorectal cancer. Clinical research studies are under process to uncover the promise of polyphenols in cancer care (Bracci *et al*., 2021). Gallic acid shows anticancer activity against prostate cancer (Tyagi *et al*., 2019), while neobavaisoflavone and rhodioflavonoside exhibit apoptosis-inducing potential (Szliszka et al., 2011). Ovarian cancer is targeted by compounds like corilagin, gallic acid, and ellagic acid, each displaying distinct anti-cancer properties (Jia et al., 2017).

While the *in-vitro* and *in-vivo* studies present promising findings, the journey from laboratory to clinical application remains complex. Polyphenols' bioavailability, metabolism and potential interactions with conventional therapies need further investigation. Developing standardized protocols, optimizing formulations, and conducting robust clinical trials will be pivotal in realizing the full potential of polyphenols in integrative cancer medicine. The diverse array of polyphenols and their demonstrated anticancer activities offer a tantalizing glimpse into the potential of integrative cancer care. These compounds, through their ability to target multiple cancer-related pathways, represent a promising avenue for enhancing conventional cancer treatments and improving patient outcomes.

## Alkaloids for Integrative Therapies

Alkaloids, a group of naturally occurring organic compounds found in various plants and organisms, have emerged as intriguing candidates for integrative therapies due to their diverse pharmacological activities. From cancer treatment to pain management, alkaloids exhibit a wide range of potential applications (Dhyani *et al.*, 2022; Kukula-Koch & Widelski, 2017). Alkaloids such as vincristine and vinblastine have long been recognized for their effectiveness in treating cancer (Defachelles *et al.*, 2021). Derived from the periwinkle plant (*Catharanthus roseus*), these alkaloids disrupt microtubule formation during cell division, arresting the growth of cancer cells (Kothari *et al.*, 2016). Similarly, camptothecin, found in the Chinese Happy Tree (*Camptotheca acuminata*), exerts its anticancer effects by inhibiting DNA topoisomerase, an enzyme crucial for DNA replication (Baranello *et al.*, 2010; Tesauro *et al.*, 2019). These alkaloids exemplify nature's arsenal in the fight against cancer and are integral to the spectrum of integrative therapies.

Alkaloids also play a vital role in pain management. Morphine, derived from the opium poppy (*Papaver somniferum*), has been a cornerstone in pain relief for centuries (Bandieri *et al.*, 2016). It acts as a potent analgesic by binding to opioid receptors in the central nervous system. Although, quinine, derived from the bark of the cinchona tree, classically used to treat malaria and an active ingredient of tonic water has initially been found to alleviate muscle cramps due to its muscle relaxant properties, however, systematic studies did not observe any significant difference between placebo versus treatment groups (El-Tawil *et al.*, 2015). Therefore, a careful evaluation of bioactive compounds for their use in integrative medicine is essential before proposing them as a treatment option. Further, the significance of alkaloids in integrative medicine is not limited to standalone applications. Combining alkaloids with other therapeutic modalities, including conventional treatments and complementary therapies, holds the potential for synergistic effects. Paclitaxel, an alkaloid derived from the Pacific yew (*Taxus brevifolia*), has the ability to stabilize microtubules complements its use in chemotherapy regimens, enhancing the efficacy of conventional cancer treatments (Reguera-Nunez *et al.*, 2019; Shetti *et al.*, 2019).

While alkaloids offer promising therapeutic avenues, challenges such as standardization, potential side effects and interactions with other medications need careful consideration. As with any integrative approach, individualized treatment plans that factor in patient characteristics and preferences are crucial. From their roles in cancer treatment to pain management and beyond, alkaloids exemplify the convergence of traditional wisdom and modern scientific understanding. Integrative medicine, with its emphasis on personalized care and comprehensive approaches, stands to benefit from the potential of alkaloids as valuable additions to the therapeutic toolkit.

## Terpenoids and Flavonoids: Versatile Compounds in Integrative Approaches

Terpenoids, aromatic compounds commonly found in essential oils and plant extracts, exhibit a wide range of biological activities. Terpenoids such as menthol and limonene are potent antineoplastic as well help in alleviating pain, reducing anxiety and promoting relaxation during cancer therapy (Fallon *et al*., 2015; Mandal *et al*., 2023). Their interactions with neurotransmitter receptors and ion channels underlie their therapeutic actions (Mandal *et al*., 2023; Xu *et al*., 2020).

Terpenoids, a diverse class of naturally occurring compounds found abundantly in various plants, have garnered attention for their multifaceted therapeutic potential within integrative medicine. With a broad range of pharmacological activities, terpenoids offer a versatile toolkit for addressing various health challenges. Terpenoids such as curcumin and limonoids exhibit potent anti-inflammatory effects (Memarzia *et al*., 2021). Derived from the turmeric root (*Curcuma longa*), curcumin modulates inflammatory pathways and oxidative stress, making it valuable for conditions marked by chronic inflammation. We have already discussed the anticancer properties of curcumin and its derivatives as a single agent and in combination with chemotherapeutic drugs. Likewise, limonoids found in citrus fruits have demonstrated anti-inflammatory actions by targeting inflammatory mediators (Shin *et al*., 2020). Additionally, terpenoids can modulate the immune system, influencing immune responses and potentially improving immunotherapy response (Lee *et al*., 2021). Carotenoids, found in colorful fruits and vegetables, are well-known terpenoids with strong antioxidant properties (Bakshi *et al*., 2022). Their ability to scavenge free radicals helps mitigate oxidative stress, a common contributor to adverse effects of chemotherapy and radiation therapy (Salek *et al*., 2021). Triterpenes and monoterpenes hold promise in cancer management (Kusmita *et al*., 2022). Triterpenoids, found in plants like licorice and ginseng, exhibit anticancer properties by targeting various signaling pathways involved in tumor growth and metastasis (Lu *et al*., 2016; Tae *et al*., 2011). Monoterpenes, on the other hand, contribute to apoptosis, inhibiting the survival of cancer cells (El Gaafary *et al*., 2019). The versatile mechanisms through which terpenoids impact cancer biology make them attractive candidates for integrative cancer therapies. Integrative oncology also focuses on neurological health and bioactive compounds such as linalool, a monoterpene found in lavender and other aromatic plants, which exert calming effects on the nervous system, potentially aiding in stress and anxiety management (Li *et al*., 2022). Ginkgolides, terpenoid lactones derived from the Ginkgo biloba tree, possess neuroprotective properties, making them intriguing candidates for cognitive health support (Hua *et al*., 2017).

Flavonoids, a diverse class of naturally occurring compounds abundant in plants, have gained recognition for their dual role in integrative medicine, encompassing both immunomodulatory and antioxidant effects (Chen *et al*., 2018). Quercetin, a widely distributed flavonoid, modulates cytokine production and immune cell function and contributes to maintaining a balanced immune response (Jing *et al*., 2021). Similarly, epigallocatechin gallate (EGCG), found abundantly in green tea, demonstrates immunomodulatory effects by influencing immune cell proliferation and activation (Pae *et al*., 2010). Anthocyanins, responsible for the vibrant colors in fruits and vegetables, scavenge free radicals and mitigate oxidative stress, thus contributing to cellular health (Ali *et al.*, 2018). This antioxidant prowess extends to catechins, such as Epigallocatechin Gallate (EGCG), found in tea leaves. By neutralizing harmful free radicals, flavonoids help combat oxidative damage and reduce the risk of chronic diseases. Flavonoids further show promise in cancer prevention and management. Genistein, a soy-derived flavonoid, exerts anti-cancer effects by influencing signaling pathways involved in tumor growth (Zhang *et al.*, 2020). Similarly, quercetin demonstrates potential in suppressing cancer cell proliferation and inducing apoptosis, contributing to its role in integrative cancer therapies (Kedhari Sundaram *et al.*, 2019). Flavonoids, particularly catechins and quercetin, have garnered attention for their potential neuroprotective effects. They exhibit anti-inflammatory actions that may contribute to cognitive health and support brain function. Additionally, flavonoids like rutin influence Brain-Derived Neurotrophic Factor (BDNF), a protein essential for neurogenesis and synaptic plasticity (Gomez-Pinilla *et al.*, 2011).

The significance of terpenoids and flavonoids in integrative medicine lies in their holistic potential. From immune support and cancer prevention to cognitive health, terpenoids and flavonoids offer a spectrum of therapeutic potential. The aromatic compounds found in essential oils are often rich in terpenoids, contributing to their diverse effects, including pain relief, relaxation and immune support (Masyita *et al.*, 2022). While these bioactive compounds offer promising therapeutic avenues, challenges such as bioavailability and individual variability must be considered. The complex interplay between terpenoids and flavonoids with other compounds, both natural and synthetic, underscores the importance of a comprehensive understanding of their interactions. From anti-inflammatory and antioxidant actions to cancer-fighting potential and neuroprotection, terpenoids offer a rich tapestry of therapeutic effects. As integrative medicine continues to evolve, the versatility of terpenoids positions them as essential players in comprehensive health approaches.

## Bioactive Compounds in Exclusive Cancer Treatment

Cancer treatment has seen significant advancements in recent years, and alongside traditional therapies a growing body of research is uncovering the potential of bioactive compounds as adjuncts or alternatives in cancer management. These compounds, derived from natural sources, exhibit diverse mechanisms of action that target various facets of cancer biology. Following are some potent bioactive compounds exclusively used for cancer treatment, highlighting their modes of action, evidence-based support and potential future directions.

### Curcumin

Curcumin, a polyphenol found in turmeric, has gained substantial attention for its potential anticancer properties. It exhibits multiple mechanisms of action, including modulation of inflammatory pathways, induction of apoptosis and inhibition of angiogenesis. Curcumin, holds immense promise in cancer therapy. It exhibits a multifaceted potential, demonstrated by its ability to inhibit key processes involved in tumor progression and metastasis (Hosseini *et al.*, 2022). Studies underscore curcumin's capability to modulate critical cellular targets like epidermal growth factor receptor (EGFR), nuclear factor  B (NF- B), Cyclin-dependent kinase-1 (CDK1), vital players in cancer development and resistance to treatments (Saeed *et al.*, 2022).

Clinical trials have investigated curcumin's efficacy in various cancers, such as breast, colorectal, and pancreatic cancer (Reviewed by (Karaboga Arslan *et al.*, 2022)). While challenges like bioavailability persist, innovative formulations and delivery methods are being explored to enhance curcumin's therapeutic potential. Mitocurcumin, a derivative of curcumin, showed improved bioavailability and alone decreased cancer stem cell population in lung cancer through increased ROS in cancer cells (Jayakumar *et al.*, 2017). Curcumin and its derivatives have also been used to target blood cancers (Zhou *et al.*, 2021). Importantly, the significant achievement of incorporating curcumins and their derivatives in cancer treatment lies in their ability to effectively inhibit drug-resistant (Bilajac *et al.*, 2022; Pacin *et al.*, 1987; Tseng *et al.*, 2021) and dormant leukemic cells (Liu *et al.*, 2020).

### Paclitaxel

Paclitaxel, derived from the Pacific yew tree, is a well-established chemotherapy agent (Pereira *et al.*, 2019; Sahin *et al.*, 2020). Paclitaxel exerts its anticancer effects by stabilizing microtubules, integral to cellular structure and division (Weaver, 2014). This leads to a prolonged metaphase arrest during cell division and triggers apoptosis due to cellular stress (Khing *et al.*, 2021). Additionally, paclitaxel possesses anti-angiogenic properties, impeding tumor vascularization (Yaniz *et al.*, 2020). However, its impact on rapidly dividing normal cells results in side effects like hair loss and gastrointestinal disturbances (Hu *et al.*, 2021; Zhu *et al.*, 2022). Despite its clinical success, efforts to improve its delivery and reduce side effects through nanoparticle-based formulations and combination therapies are ongoing (Lee *et al.*, 2012; Li *et al.*, 2019).

### Vinca Alkaloids

Vinca alkaloids, a group of chemotherapy drugs including vincristine, vinblastine and vinorelbine, play a vital role in cancer treatment by disrupting microtubule dynamics within cells. They bind to tubulin, a protein crucial for microtubule formation, inhibiting microtubule assembly and causing mitotic arrest during cell division (Dhyani *et al.*, 2022; Hoang *et al.*, 2022). These compounds have been utilized in treating various cancers, such as leukemia and lymphoma (Ballout *et al.*, 2019; Kavallaris *et al.*, 2001; Khan *et al.*, 2022). Their side effects can include peripheral neuropathy, constipation, and bone marrow suppression; however, research continues to refine their dosing regimens and explore their potential in combination therapies.

### Bacillus Calmette-Guérin (BCG)

Bacillus Calmette-Guérin (BCG) holds a dual role as a tuberculosis vaccine and an intriguing cancer treatment, with a prominent focus on non-muscle invasive bladder cancer (Chang *et al.*, 2016). When introduced directly into the bladder, BCG becomes a potent trigger for immune responses. This occurs through the activation of immune cells and the release of cytokines, fostering an environment of controlled inflammation that also contributes to the establishment of immune memory (Buffen *et al.*, 2014; McAveney *et al.*, 1994). While its efficacy in targeting cancer cells is acknowledged, the BCG therapy journey often comes with a caveat of potential side effects (Koch *et al.*, 2021).

### Arsenic Trioxide

Arsenic trioxide, traditionally known for its toxic properties, has found a surprising role in modern cancer treatment, particularly in the management of certain types of leukemia. Notably, in acute promyelocytic leukemia (APL), arsenic trioxide induces differentiation and apoptosis of immature leukemia cells (Estey *et al.*, 2006). Additionally, it promotes apoptosis, or programmed cell death, by interfering with signaling pathways critical for cancer cell survival (Chen *et al.*, 1996). Arsenic trioxide has demonstrated effectiveness, often in combination (e.g., all-trans retinoic acid) with other treatments, leading to remissions in APL cases where traditional therapies might be less effective (Huang *et al*., 2016). Though its potential side effects like heart arrhythmias and liver toxicity require careful monitoring, arsenic trioxide exemplifies the innovative repurposing of toxic compounds for targeted cancer therapies.

## Conclusion

Bioactive compounds exclusively used for cancer treatment represent a promising frontier in oncology. Their unique mechanisms of action, often distinct from traditional therapies, highlight the potential for synergistic combinations and reduced side effects. Clinical evidence continues to accumulate, refining our understanding of their efficacy and potential applications. Several bioactive compounds such as curcumin, paclitaxel, ATO and BCG have already made their way into cancer therapeutics while others such as resveratrol, flavonoids and anthocyanins are slowly making their way into integrative medicine for better patient outcomes when synchronized with standard care therapy. In summation, we have attempted to explore the symbiotic relationship between bioactive compounds and the integrative medicine landscape. As research advances and innovative formulations are developed, bioactive compounds offer a nuanced and personalized approach to cancer treatment, contributing to the evolving landscape of integrative oncology.

## Disclosure

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**REVIEW ARTICLE**

# Improved Fruit Production Through Plant Bio-Regulators –An Overview

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## Abstract

Bioregulators are compounds found in nature or produced intentionally that can control one or more particular physiological and biochemical processes in a variety of species. They most likely do this through influencing gene and enzyme interactions (Olaiya, 2013). Plant growth regulators (PGRs) or plant bio-regulators (PBRs) are chemical substances other than nutrients that, when given in small quantities. It might change a plant's physiological processes, according to Chandana and Puja (2022). Commercial fruit has previously been produced using gibberellins, cytokinins, ethylene, auxins, and abscissic acid. These five classic growth hormones having specific functions in growth and development. It is necessary to look at creative ways to deal with the problems related to fruit production and the increasing demand from consumers. One of them is to use plant bio-regulators of the next generation. These include, among others, salicylic acid, polyamines, 1-MCP, brassinosteroids, jasmonates, and prohexadione-Ca. From seedling growth to fruit quality enhancement, leaf and fruit abscission prevention, pre-harvest drop reduction, and inducing resistance to biotic and abiotic stress, these can be applied at all stages of fruit agriculture (Chandana and Puja, 2022). These new generation plant bioregulators may be used with modern biotechnological treatments to endogenously modify them and use them to create a unique variety for commercial exploration (Jia *et al*., 2015).

**Key words**:- Plant bio-regulators, Brassinosteroids, Jasmonic acid, Polyamines, Salicylic acid, 1-MCP, Prohexadione-Ca.

## Introduction

According to Olaiya (2013), bioregulators are chemicals that may be found in nature or manufactured with the objective of regulating one or more specific physiological and biochemical processes in a variety of species. They most likely do this via influencing gene and enzyme interactions. Plant growth regulators (PGRs) are organic compounds that modify physiological processes in plants, apart from nutrition. In order to regulate how plants digest food, PRGs, often referred to as biostimulants or bioinhibitors, act inside plant cells to either stimulate or block a specific enzyme or group of enzymes. They usually only become active at very low concentrations in plants (El-al and Faten, 2009). Bioregulators are substances that influence the biological responses exhibited in plant tissues. Although their effects on growth and development are known to occur at very low concentrations, at high concentrations and in the absence of any nutritive or biocidal actions, they are known to interfere with the growth and development of plants (Rademacher, 2000).They quickly enter and are absorbed by plants through the living surface of cells of most plant components. The whole life cycle of a plant, from fertilization of the egg cell to senescence and death, is regulated by bioregulators. Their significance lies in their ability to influence how plants

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respond to external stimuli and adapt to adverse surroundings. Plant defense mechanisms; abiotic stress caused by heat or cold, salinity, drought or waterlogging, nutrient deficiency or excess, pollutants, tropic, nastic movements, growth regulator biosynthesis, transport, and metabolism are among the physiological and biochemical functions of bioregulators in plants according to El-Rokiek *et al.*, (2012).

Physiological responses that are regulated/influenced by PBRs (Green *et al.*, 2010):

- Promotion of feathering and branching
- Increase flower bud formation
- Inhibit flower bud formation
- Thinning by promotion of fruit/flower abscission
- Retard pre-harvest drop
- Improve fruit finish
- Improve fruit shape
- Vegetative growth control
- Increase fruit set
- Increase fruit red colour
- Advance fruit ripening
- Delay fruit ripening
- Enhance rooting suppress growth of water sprouts

## Classes of PBRs

Different PBRs may be classified into two main groups depending on their chemical makeup: chemical-based and hormone-based. Few of these PBRs supply structural elements, while the majority of them regulate cellular redox homeostasis in order to carry out their functions.

## Chemical Based PBRs

It has been shown that a variety of physiological and non-physiological substances can increase crop production in field settings. A brief description of the mechanical underpinnings of the operation of a few popular PBRs are as follows.

## Thiourea

Thiourea, formula $SC(NH_2)_2$, is an organo-sulfur chemical. Only the substitution of the sulfur atom for the oxygen atom lessens its structural similarity to urea. The characteristics of urea and thiourea are very different. Thiourea is useful in several situations. Thiourea was first administered to plants at modest concentrations to promote dormancy (Tukey and Carlson, 1945). The cause of the impact was identified by Hendricks and Taylorson (1975) as catalase inhibition promotes NADPH-mediated $H_2O_2$ oxidation, an oxidant in the pentose pathway that yields glucose. Subsequently, it was applied as a regulator of plant development (Gul and Weber, 1998). Because of its capacity to boost plant development, stress tolerance and agricultural productivity, thiourea has gained greater attention lately (Pandey *et al.*, 2013). These advantages result from more rapid physiological translocation of compounds from source (leaves) to sink (pods) and coordinated regulation of the plant source-to-sink link (Pandey *et al.*, 2013 and Srivastava *et al.*, 2008). The capacity of thiourea to scavenge a wide variety of reactive oxygen species, including superoxide radical and $H_2O_2$ generated by biological systems, is connected to its molecular processes (Gao *et al.*, 2008). Thiourea application was used as a strategy to identify redox-regulated components linked to calcium and ABA signaling in *Brassica juncea* seed germination because redox signaling, or ROS-mediated signaling, is widely recognized as one of the key regulators of stress signaling in plants (Gilroy *et al.*, 2014). The molecular alterations caused by thiourea were positively reflected in plants' resistance to a number of environmental stresses in addition to enhanced energetics (Srivastava *et al.*, 2009), antioxidant defense (Srivastava *et al.*, 2011), plant-water homeostasis (Srivastava *et al.*, 2010b) and sulfur metabolism (Srivastava *et* *al.*, 2014). Apart from its indirect effects on ROS scavenging, thiourea has also been demonstrated to have direct effects at higher doses (in the mM range, thiourea was provided in M for the indirect effects). These include the anti-weeding, antinitrification and persistent prevention of nitrification properties of thiourea.

## Silicon

According to Ehrlich (1981), silicon (Si) is the second most prevalent element in the crust of the Earth. It follows oxygen at 47% in weight, making up 27.7% of the total weight of soil. In clay soil, it varies from 200 to 300 g Si/kg, but it reaches 450 g Si/kg in sandy soils. Its dry weight concentration in soil varies from 1 to 45%, according to Sommer *et al.* (2006). The majority of tropical and subtropical soil has low silicon levels due to continual leaching processes and extensive crop cultivation, even if the soil may be able to support a normal silicon supply. An estimated 210–224 million tons of silicon is taken from agricultural soils worldwide each year, according to FAO calculations (Meena *et al.*, 2013). Normally, polymerized soil silica is present. It is subsequently converted into water-soluble monosilicic acid or orthosilicic acid ($H_4SiO_4$), by chemical or biological processes and is assimilated by plants. A vital component of agricultural fertilizers, silicon strengthens plant's defenses against a range of abiotic stressors such as lodging, drought, radiation, heat waves, freezing temperatures and ultraviolet light, as well as chemical stressors including salt, metal toxicity, malnourishment and excess salt. Due to silicate's positive effects on plant water balance, photosynthetic efficiency, leaf erectness and xylem channel structure at high transpiration rates, most of the effects reported with silicate supplementation are caused by silicate. The Si-mediated reduction in excessive water loss is brought on by the contraction of stomatal pores. Furthermore, Si deposition in roots lessens the binding sites for various metals, including P and Mn, which lessens metal-induced toxicity in plants (Farooq *et al.*, 2013). reported that significant interaction between the redox signaling system and hormones are also involved in the Si-mediated reduction in metal load. Si reduces symptoms caused by deficiency of essential nutrients such as potassium and also decreases toxicity caused by metals. This type of dual action provides support to the theory that Si is not a simple nutrient but rather tightly regulates the signaling pathway responsible for detecting the plant demand in response to changing environmental circumstances.

## Potassium

The second most common mineral ingredient in plants, potassium (K ), is both necessary and abundant. It ranges in concentration from 0.025 to 5 mM in bulk soil solution. Simultaneously, plants normally acquire 250 A. K. Srivastava *et al.* (2011). 2-10% of K per dry weight basis or around 50–250 mM and can keep the cytosolic K concentration relatively constant, usually in the range of 100–200 mM (Britto and Kronzucker, 2008). This is made possible by the carefully planned control of an intricate web of potassium transport mechanisms. These include the KUP/HAK/KT, HKT and K /H transporters. Multiple types of potassium-permeable nonselective cation channels shaker-type and 'two-pore' potassium channels (Very and Sentenac, 2003). The various roles that potassium plays in plants are connected to its essentiality. The retention of cell turgor pressure, elongation, osmoregulation, stomata, leaf movements, tropisms, enzyme activation, phloem solute transport, cation/anion balancing, control of membrane polarization, regulation of cytoplasmic pH, synthesis of proteins and carbohydrates and energy conservation across membranes are a few of these. Besides from these widely recognized roles, K has recently been shown to play a new role as a 'molecular switch' that allows plants to regulate their growth and metabolism in the early stages of stress as well as during the recovery phase. In the morning, the cell releases K , which leads to the production of reactive oxygen species (ROS). This can be attributed to either increased activity of NADPH oxidase that is bound to the plasma membrane or by misleading electrons from the photosynthetic electron transport chain. In the recovery phase, high ROS levels trigger feedback control on the expression of transporters and K channels, which helps the cell return to K homeostasis. Severe stress can also cause a sharp drop in K , which can trigger several endonucleases and proteases which promote programmed cell death (PCD). The fate of a cell is thus determined in part by its K level, which under mild stress causes ROS to restore K homeostasis and under severe stress causes PCD to save metabolic energy for stress adaption and repair. ROS, membrane voltage, intracellular $Ca_2$, polyamines, phytohormones, and gaso-transmitters are all closely related to the regulatory functions of K . The development of K -based PBRs was prompted by the significant function that K plays as a signal regulator and fertilizer. The most common K source is $KNO_3$, while $K_2CO_3$ is also occasionally utilized (Wei *et al.*, 2013).

## Polyamines

Common low-molecular-weight aliphatic amines with a straight chain are called polyamines (PAs). Triamine spermidine ($Spd_3$), tetramine spermine ($Spm_4$), and diamine putrescine ($Put_2$), an important precursor to other PAs are among them. Their concentrations in the cell range from 0.1 to 10 mM. Because of their polycationic properties, PAs may interact with proteins, nucleic acids, membrane phospholipids and components of cell walls at physiological pH, stabilizing these molecules. Additionally, because they scavenge ROS, PAs have anti-stress properties. The recognition of PAs as a novel class of plant bioregulators has been brought about by these causes. Transgenics have been shown to exhibit increased stress tolerance due to the overexpression of several polyamine biosynthetic genes, including arginine decarboxylase (ADC, EC 4.1.1.19), ornithine decarboxylase (ODC, EC 4.1.1.17), S-adenosyl methionine decarboxylase (SAMDC, EC 4.1.1.50) and Spd synthase (SPDS, EC 2.5.1.16) using a transgenic approach. In the instances of ADC, SPDS and SAMDC, it was critical that populations evolve resistance to a range of stressors, including salinity, drought, low and high temperatures and parquet toxicity. To improve agricultural output and plant stress tolerance, several efforts are thus made to use exogenous PAs. In addition to scavenging compounds directly, PAs-mediated regulation of ROS and/or associated signaling is associated with their breakdown. The process is carried out by a flavoprotein polyamine oxidase (PAO, EC1.5.3.3), which changes Spd into 1-pyrroline, 1,3-diaminopropane and $H_2O_2$ or Put and $H_2O_2$. Among the ROS that $H_2O_2$ produces is the hydroxyl radical (OH). Hence, PAs have the ability to act as oxidants or antioxidants. They also function as an ion regulator in plants. Because it has been shown that the Spd-derived $H_2O_2$ signal induces $Ca_2$ influx to regulate pollen tube growth, *Arabidopsis thaliana* mutants lacking the expression of PAO have decreased pollen tube growth and therefore poorer seed yield (Wu *et al.*, 2010). By causing their nonselective current and passive ion fluxes, PAs can control the synergism between $Ca_2$ and K homeostasis in roots through the OH radical. However, only the root maturation zone was affected, and only the $Ca_2$ pump was demonstrated to be activated in the distal elongation zone. Additionally, OH and PAs can activate $Ca_2$-ATPase, alter H pumping, and decrease the activity of constitutively produced K+ and nonselective cation channels. It has also been shown that exogenous PAs restrict the amount of Na that enters roots and prevent K+ from leaving shoots, improving K /Na balance throughout the plants (Zhao *et al*., 2007). An efficient and well-coordinated regulation between PAs and ROS has been suggested as a major contributor to the genetic variability of salt sensitivity. Hence, PAs levels may cause a considerable alteration in the ion balance in plants.

The decarboxylation of arginine or ornithine to create putrescine in plants is catalyzed by arginase decarboxylase or ornithine decarboxylase, respectively. The addition of two aminopropyl groups to putrescine (put) results in the production of spermidine and spermine, which are catalyzed by the enzymes spermidine (SPD) synthase and spermine (SPM) synthase, respectively. S-adenosylmethionine is broken down by the enzyme S-adenosylmethionine decarboxylase to yield aminopropyl moieties.

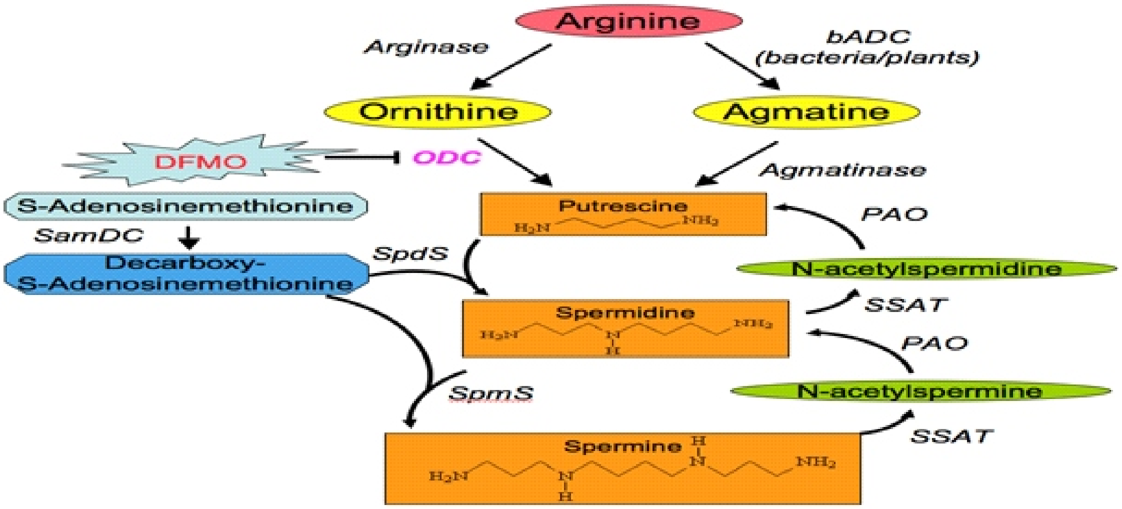

**Fig. 1 : Metabolism of Arginime to Spernine**

## Hydrogen Peroxide

Reactive oxygen species, or ROS, were first identified as toxic byproducts of aerobic metabolism that might be cleared by antioxidants and antioxidative enzymes. Examples of ROS include hydrogen peroxide ($H_2O_2$) and superoxide radial ($O_2$). Still, they are now recognized as significant secondary messengers controlling several plant functions because of the ongoing development of extremely effective ROS scavenging mechanisms. Hu *et al*., (2009) conducted tests on the exogenous application of $H_2O_2$ to improve crop yield and tolerance under various stress situations. The primary function of $H_2O_2$ is its ability to alter many signaling components linked to the control of a broad spectrum of biological activities. Effectors of calcium homeostasis, ion channels, protein kinases (MAP Kinases), transcription factors (NAC, ZAT, DREB, and WRKY), miRNAs (mir169, 397, 528, and 827) and phosphatases are among the most common targets of $H_2O_2$. Additionally, the $H_2O_2$ mediates crosstalk between nitric oxide, salicylic acid, auxin and polyamine signaling. The control of root development, which is primarily controlled by auxin and hemoglobin oxygenase is a prime example of this kind of crosstalk (Ma *et al*., 2014). According to Feng *et al*. (2012), stress stimuli often raise the amount of auxin, which activates a number of transcription factors, including lateral organ boundaries domain 16 (LBD16), LBD18 and LBD29, to promote roots. The key players in auxin action are the rate-limiting enzymes called haem oxygenases (HOs, EC 1.14.99.3), which break down hemoglobin into equimolar quantities of biliverdin IXa (BV), free iron ($Fe_2$ ), and carbon monoxide (CO). The HO $^{1}$ subfamily includes HY1, HO $^{3}$, and HO $^{4}$, whereas the HO $^{2}$ subfamily includes HO $^{2}$, which is not a real HO due to its inability to bind or decompose heme. These four distinct HO types have been found in *A. thaliana* and have been separated into two subfamilies. When $H_2O_2$ activates HOs, CO is produced, and CO has an impact on calcium and methyljasmonate signaling. According to Plant Bioregulators for Sustainable Agriculture 253 (Ma *et al.*, 2014), this also activates genes related to the cell cycle, which enhances rooting. A redox responsive transcription factor known as UPBEAT1 (UBP1) mediates the early transition in roots from proliferation to differentiation, while the clock-regulated MYB-like transcription factor KUODA1 (KUA1) controls the growth of leaf cells and is also influenced by apoplastic ROS homeostasis (Lu *et al.*, 2014). As a result, the entire mechanism of $H_2O_2$ signaling is quite complex and dependent upon several parameters, The beneficial effect of $H_2O_2$ application can also be linked to plant's acclimatization to oxidizing environments, which enables them to effectively activate their antioxidant defense system when they meet subsequent challenges. This is in addition to modifying ROS signaling.

## Nitric Oxide

Nitric oxide (NO), a redox-active gaseous molecule, is a key secondary messenger that regulates a number of physiological and developmental processes in plants. The bulk of NO's actions are caused by either myo-Inositol phosphate synthase or haem-based signaling system activation. Plant's rooting systems are improved by activating the HOs signaling system and MIPS-based signaling facilitates the synthesis of myo-inositol, a crucial molecule for cell metabolism and plant growth. Inositol is a precursor to many different compounds and derivatives of it include phytic acid (InsP6), which is used as a storage phosphate, cell wall biogenesis, auxin physiology control, membrane biogenesis, signal transduction and stress tolerance. NO has a major regulatory role in root feeding, metabolism and iron homeostasis. Through activating the transcription factor FIT (FER-like iron deficit induced transcription factor), it regulates the activities and turnover of iron reductase (FRO) and iron transporter (IRT) by controlling the expression of their genes. According to Chen *et al.* (2013), maintaining K homeostasis under stressful conditions requires not only iron but also NO-mediated signaling. Furthermore, through the activation or inactivation of NADPH oxidase, NO levels may mitigate the degree of oxidative damage in plants. Applying NO exogenously in the form of sodium nitroprusside as a nitric oxide donor has been demonstrated to magnify the stress that plants undergo from a variety of abiotic stimuli as a result of these several effects.

## Hydrogen Sulfide

Hydrogen sulfide ($H_2S$) is a well-known environmental toxin that is famous for its ability to inhibit mitochondrial cytochrome oxidase. However, it is also known to function as a signaling molecule in plants where it is produced at suboptimal concentrations. In fact, after NO and CO, $H_2S$ is now regarded as the third most important gasotransmitter. Endogenous $H_2S$ can have a bidirectional effect on the redox state of cells. It can also concurrently oxidize the environment by increasing the availability of reduced sulfur to enable greater glutathione production and a reduced environment by enhancing the activities of glucose-6-phosphate dehydrogenase and plasma membrane NADPH oxidase. The exact role of $H_2S$ varies depending on the requirements of the plant, although it has recently been called a 'referee' to ensure that ROS machinery functions properly in a range of developmental phases and changing environmental circumstances (Hancock and Whiteman, 2014). Due to its dynamic nature, $H_2S$ is a valuable tool for regulating plant responses to a range of abiotic stresses and environmental pollutants. Supplementing sodium hydrosulfide (NaHS, source of $H_2S$) decreased the cadmium influx in Populus cells via activating plasma-membrane bound calcium channels with $H_2O_2$. i.e. Furthermore, a variety of stressors that cause oxidative damage in plants have been demonstrated to be ameliorated by NaHS. $H_2S$ functions subsequent to NO-mediated signaling, according to recent studies. Thus, a better knowledge of the therapeutic effects of NO and $H_2S$ requires an understanding of the $H_2S$-NO interaction.

## Hydrogen-Rich Water

Hydrogen is the most abundant and lightest chemical element in the universe, making up over 75% of its elemental mass. Hydrogen gas ($H_2$) has been demonstrated in a growing number of studies to selectively reduce hydroxyl radicals, which reduces acute oxidative stress in a range of animal models. The recommended method for treating $H_2$ is presently hydrogen-rich water (HRW), which has a high concentration of hydrogen and is used because concentrations of $H_2$ higher than 5% can cause explosive combinations with air. Due to its redox-active characteristics, $H_2$ is believed to have a wide range of actions. It has recently been shown to be an important gaseous molecule in plants. Plants treated with HRW are protected against oxidative stress caused by paraquat, salt and heavy metals by activating the cellular antioxidant defense mechanism through the zinc-finger transcription factor (ZAT10/12). The well-known heme oxygenase-1/carbon monoxide pathway is another method that HRW pretreatment promotes roots in cucumber seedlings. Furthermore, $H_2$ application is becoming more and more popular for usage in outdoor settings because of its tasteless, odorless, colorless, nontoxic, and inert qualities.

## Hormone Based PBRs

A wide-range of hormone based PBRs are being used in both horticulture and agriculture crops. Brief information about their mode of action is described further.

### 1. Brassinosteroid

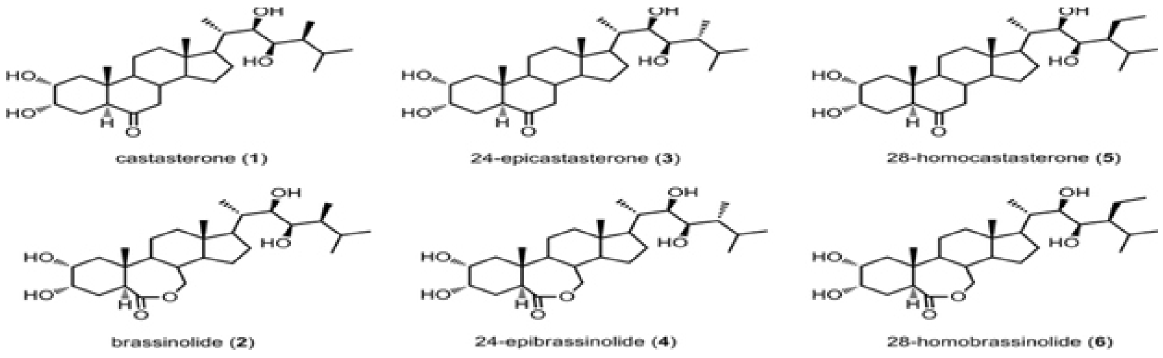
castasterone (1)
24-epicastasterone (3)
28-homocastasterone (5)
brassinolide (2)
24-epibrassinolide (4)
28-homobrassinolide (6)

**Fig. 2 Various Brassinosteroid**

Plant activities such as growth, differentiation, elongation of the roots and stems, development of pollen tubes, bending and epinasty of leaves, xylem differentiation, and tolerance to biotic and abiotic stimuli are all known to be regulated by a class of steroid hormones called brassinosteroids (BRs). More than seventy distinct compounds that make up BRs are distributed across the plant world. Researchers like Zhu *et al.* (2013) reported substantial advancements in our understanding of the mechanism behind BR production and the process of signal transduction. In short, leucine-rich repeat (LRRs), receptor-like kinase BRI1 (brassinosteroid insensitive-1) and BAK1 (BRI1-associated receptor kinase-1) collaborate to perceive BR signals. In order to ensure that the homodimer stays dormant in the absence of BRs, BKI1 (BRI1-kinase inhibitor 1) functions as a negative regulator in conjunction with BRI1. BKI1 is phosphorylated and dissociated by BRI1kinase activity, which is somewhat increased when BRs are present. BRI1 and BAK1 are transphosphorylated one after the other when BKI1 is eliminated. The two main transcription factors of BR signaling, BZR1 (brassinazole resistant 1) and BES1 (BRI1-EMS supressor 1), accumulate as a result of this activation of BR signaling kinases (BSKs). Osmolytes and antioxidant capacitance accumulate due to the activation of a number of genes by the entry of the BZR1 and BES1 into the nucleus. Furthermore, active BR signaling can boost NO generation and regulate $H_2O_2$ synthesis by upregulating the expression of NADPH oxidase. The development of stress tolerance may be promoted by any of these mediators. Auxin, ABA, polyamines, salicylic acid and other plant hormones are tightly linked to the BR-mediated signaling, according to Choudhary *et al.* (2010). In order to increase plant stress tolerance and agricultural output, boosting the endogenous BR level has been frequently used. These biotechnological techniques have been widely employed, as has the exogenous administration of BR compounds such as brassinolide. In addition, BRs have been utilized in combination with polyamines or salicylic acid (Hayat *et al.*, 2012) to increase their effectiveness in reducing a variety of abiotic stressors. Brassinolide biosynthesis is supposed to take place via two pathways from campesterol:

- Early C-6 oxidation pathway
- Late C-6 oxidation pathway

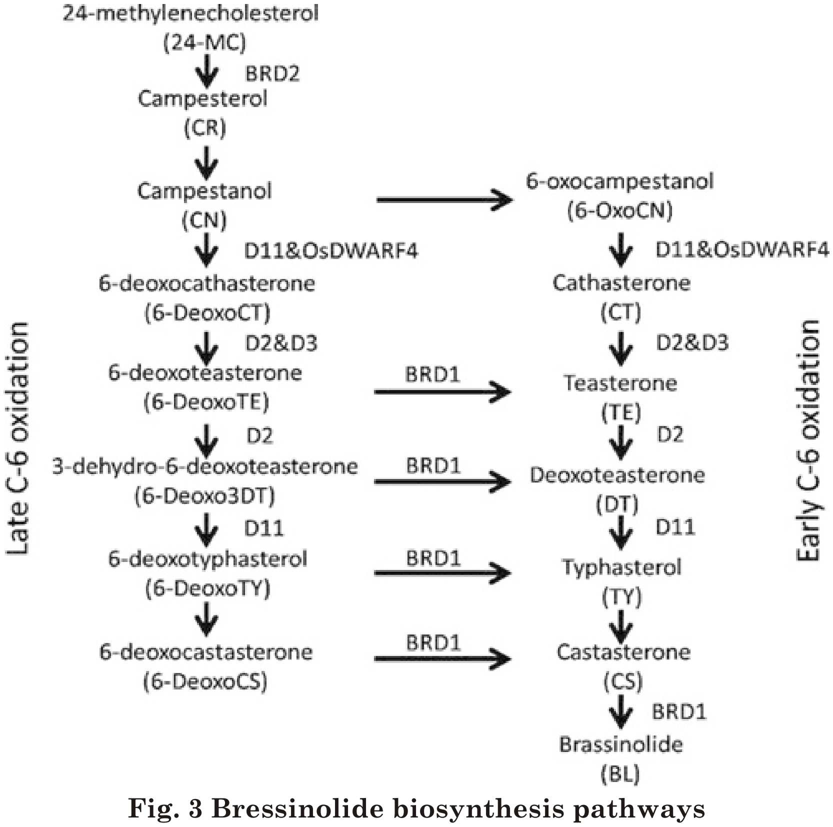

**Fig. 3 Bressinolide biosynthesis pathways**

## Salicylic Acid

Despite being used for almost 200 years as a human medicine, salicylic acid (SA, o-hydroxy benzoic acid) is a phenolic compound whose role as a plant hormone was only established 20 years ago. Numerous plant species have it in large quantities. Both the phenyl-propanoid route in the cytoplasm and the isochorismate pathway in chloroplasts may be used to make it. It can then be methylated or glucosylated to produce SA glucoside (SAG) or methylsalicylate (MeSA). SA has an inactive storage form called SAG, but its volatile derivative, MeSA, is essential for generating systemic acquired resistance. SA, on the other hand, is now well recognized as an essential signal transducer that controls plant tolerance to a variety of abiotic stimuli, including rain, drought, salt and cold. Plant resistance against pathogens was the original reason SA was discovered. SA signaling's primary transcriptional regulator is NPR1, a gene that is not expressed in *Arabidopsis*. This protein, which is redox sensitive, creates an oligomer by forming a disulphide link. Transcription is activated during SA activation when these oligomers are reduced and transported to the nucleus. Via proteasome-mediated degradation, NPR1 levels are regulated in the nucleus. SA not only modifies the function of a variety of key proteins by signaling, but also through direct interactions. In order to reduce the accumulation of ROS, for example, SA improves the performance of photosystem II and mitochondrial alternative oxidase. According to Durner and Klessig (1996), SA stabilizes $H_2O_2$ by inhibiting the activity of antioxidant enzymes such as catalase and ascorbate peroxidase. A fascinating example of hormone-redox crosstalk is SA signaling, which is similar to BA signaling but inhibits plant development because SA adversely affects cyclin D3 (CYCD3) synthesis, which starts the G1/S phase transition, whereas BA promotes growth. Transgenic *A. thaliana* lines with low SA levels have grown more quickly, which serves as evidence of this. However, these SA effects are mostly crop-specific. In contrast to *Arabidopsis*, Populus's intrinsic elevation of SA solely affects the partitioning and intake of carbon. Because of the reduction in antioxidant capability brought on by elevated SA levels, senescence is also caused by growth inhibition. Therefore, a thin line between SA's beneficial and detrimental effects must be established, and this line is dependent only on the crop and development stage. For different plants, the optimal amount of SA has been determined to reduce the degree of stress tolerance, modify flowering, and boost crop yield.

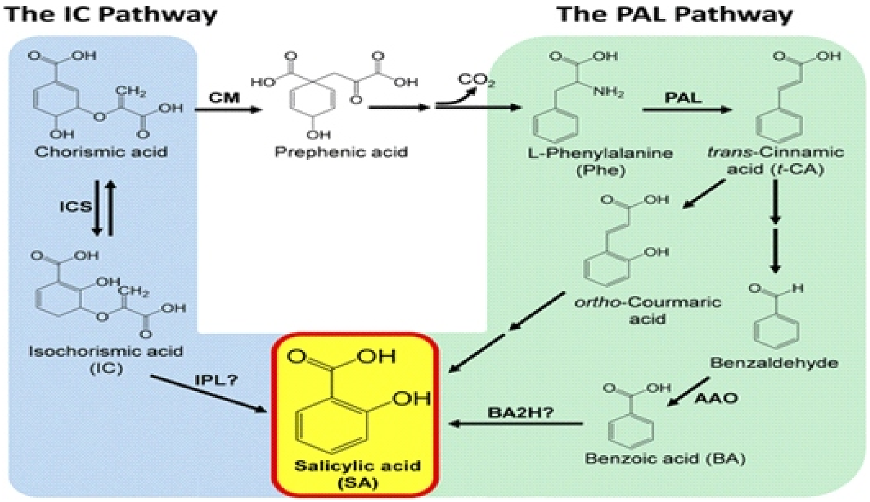

Fig. 4 Salicylic Acid biosynthesis. pathways

## Jasmonates

In chloroplast membranes, linolenic acid is converted into oxygenated fatty acids, which results in these compounds. Under the trade name MeJA, they were initially extracted from jasmine oil (*Jasminum grandiflorum*) in 1962 after being discovered as a secondary metabolite in plants. JA was subsequently isolated using *Lasiodiplodia theobromae*, a pathogenic fungus (Alridge *et al.*, 1971). The function of it in plant defense was originally shown by Farmer and Ryan (1990). Younger leaves, blossoms and reproductive tissues have the highest concentration, whereas roots and adult leaves have the lowest. Jasmonic acid (JA), methyl jasmonate (MeJA) and n-propyl dihydro-jasmonate (PDJ) are a few examples.

As aromatic elements of jasmine essential oils, methyl jasmonate and cis-jasmine are well known in the perfume business.

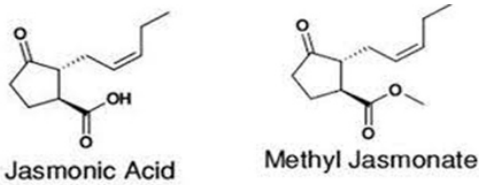

**Fig. 5 Jasmonates**

Jasmonates are normally present in plants at concentrations of 0.01-3.0 ng/g fresh weight, however *Artemisia tridentate* has been shown to have up to 95 g/g MeJA (Preston *et al.*, 2004). Linolenic acid is the starting point for the Octadecanoid route, which leads to 7-epi-JA and its conjugates and isomers.

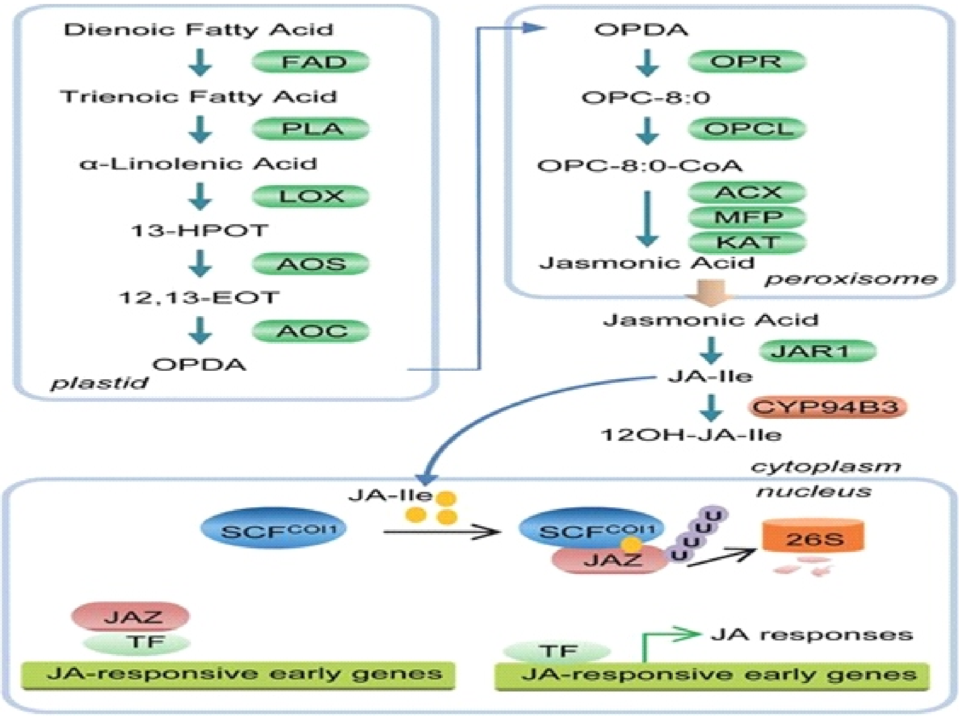

**Fig.6 Jasmonic acid metabolism**

A simplified perspective on the signaling and biosynthesis of jasmonate (JA). In plastids, peroxisomes and the cytoplasm, JA biosynthetic enzymes produce JA-Ile, which CYP94B3 can inactivate. The JA ZIM-domain (JAZ) family proteins are ubiquitinated and degraded by the 26S proteasome as a result of the interaction between JA-Ile and Coronatine Insensitive1 (COI1); consequently, downstream transcription factors (TFs) are de-repressed, which permits them to activate JA-responsive early genes and JA responses.

### Role of Jasmonic acid (JA) in plants

- Regulates plant growth and development
- Induces tuberization

- Defense responses against environmental stress
- Inhibit growth
- Inhibit germination
- Promote Senescence
- Promote pigmentation
- Flower development, leaf abscission
- Response to wounding in plants
- Transcription
- Decrease low temperature injuries
- Regulates ethylene biosynthesis and influence aroma volatile
- Systemic acquired resistance

## 1-Methyl Cyclo Propane

A synthetic plant growth regulator is 1-Methylcyclopropene (1-MCP), a derivative of cyclopropene. It is used commercially to delay fruit ripening and maintain the freshness of cut flowers. It has structural similarities with the naturally occurring plant hormone ethylene. 1-Methallyl chloride and phenyllithium, which serves as a base, react to produce methylcyclopropene:

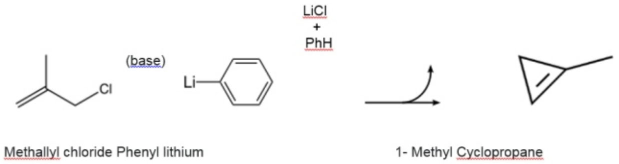

**Fig. 7 Synthesis of 1-mythyl cyclo propane**

Lithium halides should be absent from the phenyllithium. Cyclopropylbenzene is the product of the equivalent reaction between phenyllithium main and allyl chloride. The term methyl cyclopropene can relate to either of the two isomers: 1-methyl cyclopropene, which is the subject of this article, or 3-methyl cyclopropene, which is not. 1-methyl cyclopropene should not be referred to as 2-methyl cyclopropene. Additionally, keep in mind that methylcyclopropane, a cycloalkane ($C_4H_8$), is an additional distinct chemical substance. At trace levels (usually a few tenths to a few thousand ppm in the gas atmosphere), ethylene acts on a variety of plant processes, including the ripening of climacteric fruit, the opening of flowers (dehiscence process) and the shedding of leaves (abscission process). 1-MCP works by firmly attaching itself to the plant ethylene receptor, which prevents ethylene's actions (competitive inhibitor). In order to keep decorative plants and flowers fresh and stop fruit from ripening, MCP is used commercially. Coolers, shipping containers, greenhouses, truck trailers and storage facilities are among the enclosed spaces where it finds application. 1-MCP was authorized by the US Environmental Protection Agency in 1999 for use on ornamental crops under the trade name EthylBloc. 1-MCP halts or postpones wilting, leaf yellowing, opening, and mortality in cut flowers, potted flowers, bedding, nursery and foliage plants. In order to stop or delay the natural ripening process, producers, packers, and shippers in the agriculture sector utilize 1-MCP under the trade name SmartFresh. Approved and recognized for usage in more than 34 countries, including the US and the EU, 1-MCP is used in agricultural goods such as apples, kiwifruit, tomatoes, bananas, plums, persimmons, avocados and melons. The client may, however, be buying fruit that is older than anticipated despite the advantages of cheaper and fresher food. A crop protection method called MCP is also being developed. Growing field crops may be shielded from mild heat and drought conditions by applying 1-MCP to them during stressful times.

## Prohexadione- Ca

$Ca^2$ and prohexadione$^2$ ions are present in equal amounts in a calcium salt known as prohexadione-calcium. It regulates plant development and acts as an anti-lodging agent in small-grain cereals. It has the ability to control plant development, prevent the synthesis of gibberellin and act as an agrochemical. This substance is a prohexadione$^2$ . A topically applied plant regulator called prohexadione calcium inhibits the formation of gibberellin, a hormone that naturally exists in plants, hence slowing down vegetative development. The shoot internodes are notably shortened by it. Apogee in apple and pear trees lessens the need for pruning, allows more light to reach the tree canopy, improving the fruit's color and lessens the incidence of fire blight, a bacterial disease that plagues apple and pear trees. Baseline in peanuts promotes mechanical harvesting while preventing vegetative development. After mixing the components with water, ground spray equipment is used to apply the dry flowable materials to wet leaf surfaces. With maximal treatment rates of 0.375 pounds per acre per year and 1.7 pounds per acre per year, respectively, Baseline and Apogee both have 75% active ingredient.

- Carboxylic group.
- Anti- gibberellin.
- It is a structural mimic of 2- oxyglutaric acid and ascorbic acid.
- Reduces longitudinal shoot growth by blocking dioxygenases, involved in biosynthesis of gibberellin.
- Reduces ethylene formation.
- Reduces alternate bearing.
- Alternative for paclobutrazol.

## Plant Mediated Action Unified Mechanism for PBR

Although the exact mechanism of enhanced stress tolerance and agricultural productivity is PBR-specific, a universal process based on the concept of cellular redox state may be hypothesized. This is primarily justified by the fact that all PBRs aside from Si, which provides structural components either directly reduce ROS levels by functioning as scavengers of ROS or indirectly do so by interacting with enzymes that produce or scavenge ROS. The cellular redox status and associated signaling are impacted by this. Redox state is defined as the integrated ratio between the oxidized and reduced forms of redox couples occurring inside the cell. It is influenced by the level of individual ROS, ROS-producing enzymes, antioxidants, and their oxidized forms as well as their oxidation/reduction states. According to Srivastava *et al*. (2015), most stimuli alter the equilibrium of ROS in plants, even if they have different effects. The establishment of ROS homeostasis through the external application of PBRs helps to enhance the effectiveness of plants and allows them to grow to their full potential. ROSs are thought to be the central or core regulator of signaling, and they are crucial for controlling many different plant processes. Three major plant processes are mainly affected by this: increased photosynthetic efficiency and source to sink translocation to support crop output, improved root growth to facilitate enhanced water and nutrient absorption and improved metabolism to support overall plant development. The working model for the PBRs-mediated activity is depicted in the figure below. The first evidence of the redox state mediated control of root development and differentiation came from Foreman *et al*., (2003), who demonstrated that baseline ROS is necessary for root cell proliferation. It was eventually discovered that auxin was a crucial hormone regulating root formation. This is the current paradigm of auxin-mediated signaling to promote root development (see figure below). Targeted degradation of transcriptional repressors belonging to the auxin/indole-3-acetic acid (Aux/IAA) protein family is a component of auxin signaling. These repressors are frequently linked to the auxin response factor (ARF) family of transcription factors, preventing any transcription from taking place. Auxin serves as a molecular glue to maintain the connection between Aux/IAAs and transport inhibitor response1 (TIR1) or other closely related auxin signaling F-box family proteins (AFB). TIR1 and AFBs are alternate subunits of the SCF (Skp1-Cul1/Cdc53-F-box)-type E3 ubiquitin ligase, which targets the degradation of Aux/IAAs via the 26S proteosome. Several genes linked to rooting are transcriptionally activated when Auxin-induced breakdown of Aux/IAAs forms ARF-ARF dimers. The short-term redox state shift caused by NO production toward the oxidizing end may increase auxin responsiveness. TIR1 is nitrosylated by NO, which improves its ability to interact with Aux/IAAs. Ascorbate peroxide-1 (APX-1) may likewise be denitrosylated and rendered inactive by auxin, which has the ability to control redox state. Thus, a prolonged auxin concentration may result in an $H_2O_2$ buildup that would impede root growth. The transcription factor UPBEAT1 (UPB1), which controls the production of a set of peroxidases in a redox-state dependent way, also plays a role in the hormone-independent redox-mediated regulation of root development. Thus, for healthy root development, careful balancing of the redox and auxin status is needed. The majority of abiotic stressors produce redox imbalance, which hinders root growth. Using PBRRs decreases these impacts by minimizing the redox state change from the baseline condition. This preserves root development, which improves the plant's water and nutritional status and allows for the growth of plants even in challenging environments.

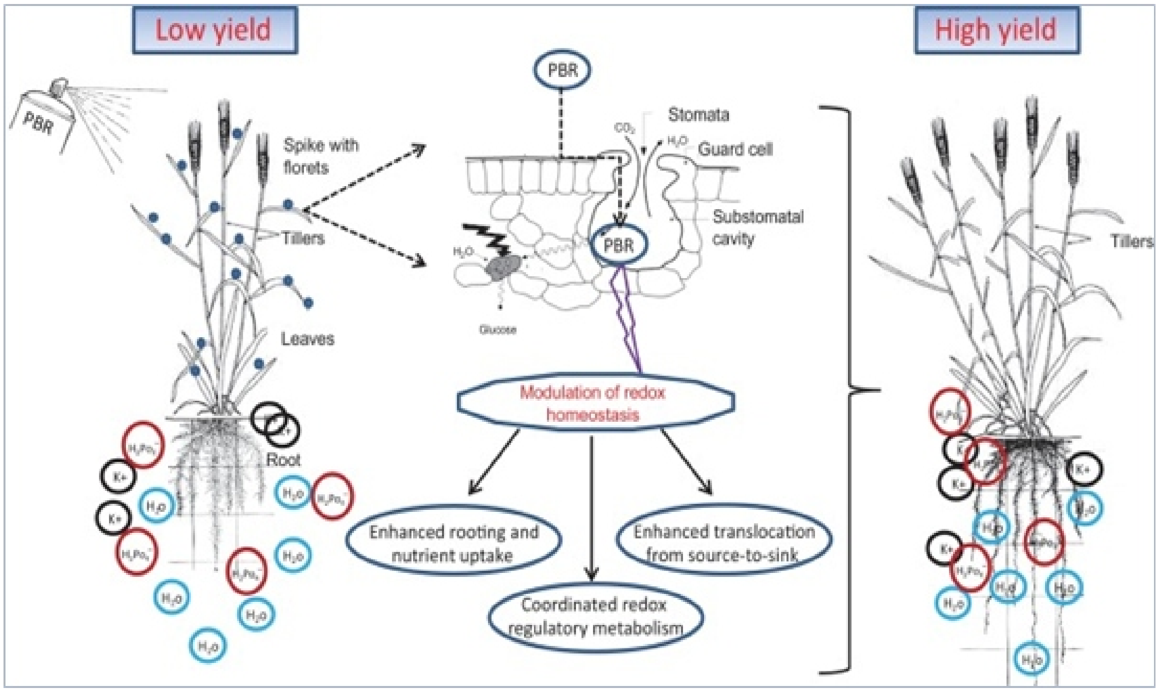

**Fig. 8 Field improvements by PBRS**

PBR administration regulates not only the root but also the aerial parts of redox equilibrium. One of a plant's first reactions to several abiotic stresses is the closure of its stomata. Through coordinated ABA and $H_2O_2$ mediated signaling, transpirational water loss is reduced. However, in stressful conditions, this inhibits gas exchange, which causes ROS to build up and oxidize, which prevents photosynthesis from occurring. Using PBR improves the source-sink connection and photosynthetic efficiency because plants are able to establish redox equilibrium. According to recent proteomic and biochemical research, most Calvin-Benson cycle enzymes and regulatory proteins can regulate redox through post-translational modifications such nitrosylation and glutathionylation. Trise phosphates, also known as glyceraldehyde-3-phosphate or Gly-3-P, are either stored in the chloroplast to aid in the production of starch or they are transported into the cytosol to aid in the synthesis of sucrose. Enzymes fructose-1, 6-bisphosphatase (FBPase) and sucrose phosphate synthase (SPS) convert gly-3-P to sucrose in the cytosol. One of the key rate-limiting components of the starch biosynthesis pathway is the redox-regulating enzyme ADP-glucose pyrophosphorylase (AGPase). The plant should keep its greater SPS and lower AGPase activity to preserve source strength. Additionally, in order to prevent feedback inhibition, the freshly produced sucrose needs to be translocated into the vacuole or toward the sink. It's been shown that redox dependence governs the majority of the transporters engaged in metabolite translocation from source to sink. Transported sucrose must first be broken down by sucrose synthase (SuSy) or invertases (INVs) in order to maintain a low sucrose level. Starch must then be produced by AGPase in order to increase sink strength. Invertases produce hexoses, which are necessary for sending out signals that set up young sinks, which are necessary to boost yield since they have the power to adjust ROS levels. In order to boost crop yield and oil content in *B. juncea*, thiourea, a PBR that is often used for agricultural plants, has recently been demonstrated to be beneficial in coordinating the various processes of photosynthesis and the source-sink link. Analogous results have also been obtained with alternative PBRs. Plant Bioregulators: A Sustainable Approach to Agriculture 263 Plants are also fundamentally composed of oxidation-reduction-based redox processes. Chloroplasts and mitochondria are where the majority of these activities occur. Light energy is absorbed in the chloroplast by photosystems I (PSI) and II (PSII), which are located in the thylakoid membrane. Linear electron flow in these photosystems results in the transport of electrons from water to NADP. Consequently, a trans-thylakoid proton gradient causes Fo F1-ATPase to synthesis ATP, while NADPH is generated as a reducing power. Electrons are subsequently transported to ferredoxin (Fdx) at the stromal side of PSI, in addition to the membrane. Fdx-NADP-reductase (FNR) disperses electrons to NADP in order to generate NADPH or directly to certain stromal activities, including the synthesis of fatty acids and chlorophyll, the absorption of S and N and reactions related to chloroplast redox control (Hanke and Mulo, 2013). Additionally, Fdx electrons can be transported to thioredoxins (Trxs) via Fdx-Trx-reductase (FTR). Small regulatory proteins called Trxs have a redox-active disulfide group that regulates the thiol-disulfide exchange of target proteins. These processes are linked to various plastidial processes, including the Calvin-Benson cycle, starch synthesis and degradation, lipid synthesis, nitrate assimilation, chlorophyll synthesis, and antioxidant metabolism. PS I can also have cyclic electron flow, as opposed to linear transfer, to create ATP without producing NADPH. Here, instead of being dispersed to stromal components, electrons from Fdx are reintroduced into the electron transport chain. Johnson (2011) states that the NADH dehydrogenase-like complex or the proton-gradient-regulation-5/proton-gradient -regulation-like protein 1 complex (PGRL1) control this cyclic flow. As Fdx-PQ reductase in *Arabidopsis*, the function of PGRL1 has been shown. It's an active redox enzyme that Trx m4 controls. To maintain the ideal redox and energy status in plants, a very careful balance between cyclic and linear electron flow is needed. The mitochondrial activity of plants, which is primarily powered by the breakdown of sucrose, also determines their energy status. In cytosol, sucrose is first degraded into pyruvate and then through TCA cycle pyruvate is completely oxidized to $CO_2$.

The electrons are moved to NAD and FAD, where they phosphorylate some ADP directly and produce NADH and $FADH_2$, respectively. The majority of the related enzymes' in vitro activity have also been demonstrated to be redox-sensitive, and the ratio of mitochondrial NAD to NADH is essential for regulating the flow of pyruvate through the TCA cycle. The photorespiratory amino acid metabolism of cysteine, proline, branched chain amino acid, lipid, and vitamin is one of the other redox-dependent processes of mitochondria, in addition to the TCA cycle. The primary locations for ROS generation are mitochondria and chloroplast, which also include a variety of enzymatic and nonenzymatic antioxidants in addition to a broad spectrum of redox processes. As a result, by taking part in redox signaling, they can regulate other plant systems. Plant hormone biosynthesis, which produces auxin and jasmonic acid (JA), is one such process. The precursor 12-oxo-phytodienoic acid (12-OPA), derivatives of JA, and JA itself make up the JA hormone family. Chroplasts create 12-OPA, which is subsequently transported to peroxisomes where it is converted to 3-oxo-2-(29-Z-penenyl)cytopentane-1-octanoic acid. JA is then formed by -oxidation three times. Peroxisomes also play a role in the conversion of IBA to IAA, which is the main type of auxin. According to Strader *et al.*, (2011), the metabolic route for IBA is projected to be similar to that of fatty acid -oxidation. The redox-regulatory metabolic network is either directly or indirectly integrated with the underlying signaling because the majority of developmental changes and abiotic/biotic stressors are linked to changes in cellular energetics and hormone levels. Therefore, our unified mechanism for PBRs mediated action which asserts that PBRs application enhances the plant's innate ability to fine-tune their redox homeostasis clearly supports the fact that most stress conditions cause redox imbalance, which can affect a wide range of plant processes. This leads to improved coordination of signaling underlying root growth, photosynthetic efficiency, source-sink relationships, cellular energetics and hormone biosynthesis, with enhanced plant growth and crop yield as the end product. Recently, such a concept of exploiting ROS/ redox based mechanisms for biofortification and crop protection has also been proposed (Kerchev *et al.*, 2015).

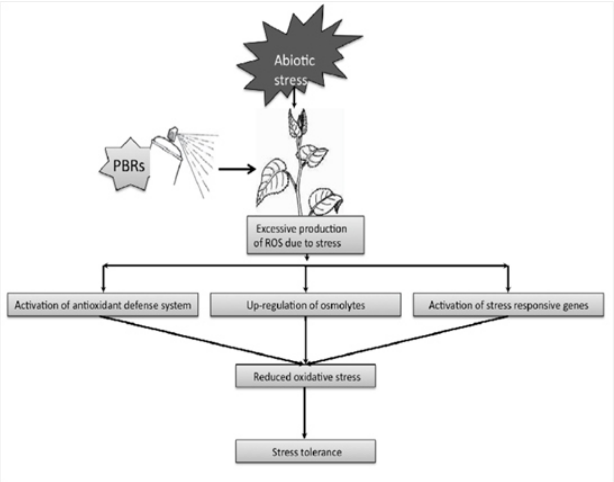

**Fig. 8 Pictograph depicting effect ofPBRs on stress tuderance**

## Conclusion and Suggestions for the Future

Multiple chemical- and hormone-based PBRs have been used in field settings to increase the stress tolerance and yield of various crops. It has been suggested that PBR's capacity to trigger redox-mediated signaling to promote roots, sustain high photosynthetic efficiency and source-to-sink translocation for greater yield and metabolism for overall plant development serves as the unifying regulatory point for this multifaceted process. In addition to different quality controls on soil, human health and grain nutritional quality, extensive assessments of PBR in different agroclimatic zones are required to enable their usage on a broader scale. Only small-scale field trials or experiments at the seedling stage have been used in the bulk of PBR investigations to yet. Plant redox signaling fundamental research has to be enhanced in order to increase the efficacy of PBRs. Subsequent investigations might concentrate on creating technology for mixed or melange-based bioregulators, which combine many bioregulators to better manage multistress situations given the unstable and ever-changing environmental conditions. Utilizing PBRs excessively may be harmful to plants because they alter ROS-mediated signaling. Our farmers need to be taught how to use PBRs sensibly according on their stress levels in order to avoid this. Developing nanotechnology-based formulations that are slow-acting and maintained over extended periods of time can enhance PBR's delivery mechanism. Plant redox equilibrium can also be maintained under stress by testing the ability of naturally occurring microbial organisms that produce antioxidants. Consequently, it could be possible to create biotic PBR that is both reasonably priced and ecologically sound. The ultimate objective of PBR-based research is to create a highly flexible and potent technology that can be applied directly to farmer fields to ensure sustainable agriculture, however this study is still in its early stages and has lots of potential for additional exploration.

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**REVIEW ARTICLE**

# Nanoparticles for Controlling Fungal Diseases in Wheat : A Comprehensive Review

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## Abstract

Fungal diseases pose a significant threat to wheat production globally, impacting both yield and quality. In recent years, the use of nanoparticles has gained attraction as a potential method for controlling fungal diseases in wheat. This review paper aims to comprehensively examine the utilization of nanoparticles in managing fungal diseases, particularly focusing on wheat crops. The paper explores the mechanisms of action, effectiveness, potential environmental impacts, and prospects of nanoparticle-based treatments for fungal disease control in wheat.

**Keywords:** Nanoparticles, Environmental impacts, Wheat, Fungal Disease.

## Introduction

Wheat is one of the most important cereal crops in the world, providing food and nutrition for billions of people. However, wheat production is threatened by various biotic and abiotic stresses, among which fungal diseases are the major cause of yield loss and quality reduction. Fungal pathogens that infect wheat include *Fusarium*, *Rhizoctonia* and *Puccinia*, which cause diseases such as **Fusarium** head blight, root rot, and rust, respectively. These diseases not only affect the quantity and quality of wheat grains but also pose a risk to human and animal health due to the production of mycotoxins and allergens. Therefore, there is an urgent need to develop effective and eco-friendly strategies to protect wheat from fungal infections.

Current challenges in managing fungal diseases in wheat include the limited availability and efficacy of resistant cultivars, the high cost and environmental impact of chemical fungicides, and the emergence and spread of fungicide-resistant strains of fungal pathogens. Moreover, the changing climate and agricultural practices may create favorable conditions for the development and dissemination of fungal diseases, increasing the pressure on wheat growers and consumers. Thus, there is a need to explore alternative and innovative approaches for controlling fungal diseases in wheat, using the advances in science and technology.

Nanotechnology is an emerging field that offers new opportunities and challenges for agriculture and plant protection. Nanoparticles are materials with at least one dimension in the range of 1-100 nm, which exhibit novel physical, chemical, and biological properties due to their small size, large surface area, and high reactivity. Nanoparticles have been widely used in various fields, such as medicine, electronics, energy, and the environment, due to their potential applications and benefits. Recently, nanoparticles have also attracted attention as a novel and eco-friendly strategy to control fungal diseases in plants, especially in wheat .

In this review paper, we aim to provide an

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overview of the current status and future perspectives of nanoparticles for controlling fungal diseases in wheat.

## Nanoparticles in Fungal Disease Control

Nanoparticles are a promising nanotechnology for controlling fungal diseases in wheat, as they have various mechanisms of action and effects on fungal pathogens and wheat plants. We will review the mechanisms of action of nanoparticles against fungal pathogens, the types of nanoparticles used for controlling fungal diseases in wheat, and the comparative analysis of different types of nanoparticles and their efficacy.

The mechanisms of action of nanoparticles against fungal pathogens are not fully understood, but several hypotheses have been proposed based on the available evidence. These are as follows:

- Nanoparticles release ions and bind to certain protein groups, which affect the function of essential membrane proteins and interfere with cell permeability.
- The nanoparticles inhibit the germination of the conidia and suppress their development.
- The nanoparticles generate reactive oxygen species (ROS) and free radicals, which cause oxidative stress and damage to the fungal cell membrane, cell wall, proteins, nucleic acids, and enzymes.
- The nanoparticles interfere with the fungal spore germination, hyphal growth, and biofilm formation.
- The nanoparticles modulate the plant immune system and induce the expression of defense-related genes.
- The nanoparticles deliver other antifungal agents, such as natural products, synthetic compounds, or biological agents, in a controlled and targeted manner.

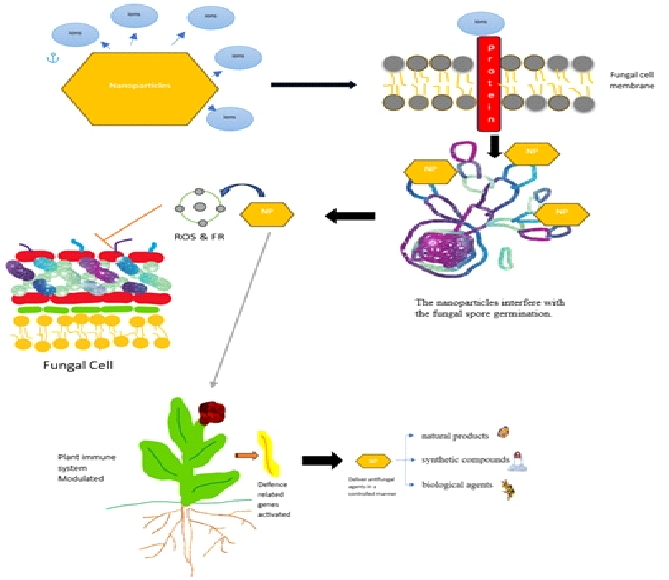

**Fig.1.** Hypothesis of mechanism of action of nanoparticles against fungal pathogens.

The types of nanoparticles used for controlling fungal diseases in wheat include metal, metal oxide, and carbon-based nanoparticles. Among these, metal nanoparticles, such as silver, copper, zinc, and titanium, have been extensively researched for their antibacterial and antifungal properties, and are known for their antiviral properties (Raliya and Tarafdar, 2014). Metal oxide nanoparticles, such as zinc oxide, copper oxide, and titanium dioxide, have also shown antifungal activity against various fungal pathogens that affect wheat. Carbon-based nanoparticles, such as carbon nanotubes, graphene, and fullerenes, have also been explored for their antifungal potential, but their applications are still limited (Cruz-Luna *et al.*, 2021).

The comparative analysis of different types of nanoparticles and their efficacy for controlling fungal diseases in wheat is not straightforward, as many factors influence the antifungal activity and performance of nanoparticles, such as the size, shape, surface charge, coating, concentration, exposure time, and environmental conditions of the nanoparticles, as well as the type, strain, and virulence of the fungal pathogens, and the type, variety, and stage of the wheat plants (Baig *et al.*, 2021). However, some general trends and observations can be made based on the available literature and research. These are as follows:

- Smaller-sized nanoparticles have higher antifungal activity than larger-sized nanoparticles, due to their larger surface area, higher reactivity, and better penetration into the fungal cells.
- Spherical-shaped nanoparticles have higher antifungal activity than other shapes, such as rods, cubes, or triangles, due to their lower aggregation, higher stability, and better dispersion in the medium.
- Negatively-charged nanoparticles have higher antifungal activity than positively-charged or neutral nanoparticles, due to their electrostatic repulsion with the negatively-charged fungal cell membrane, which prevents the attachment and internalization of the nanoparticles.
- Coated nanoparticles have higher antifungal activity than uncoated nanoparticles, due to their enhanced stability, solubility, biocompatibility, and specificity, as well as their reduced toxicity and aggregation.
- Higher concentrations and longer exposure times of nanoparticles have higher antifungal activity than lower concentrations and shorter exposure times, due to their increased contact and interaction with the fungal cells.
- Different environmental conditions, such as pH, temperature, light, and humidity, can affect the antifungal activity and performance of nanoparticles, by altering their physicochemical properties, stability, and bioavailability.
- Different fungal pathogens have different susceptibility and resistance to nanoparticles, depending on their species, strain, and virulence, as well as their cell wall and membrane composition, structure, and function.
- Different wheat plants have different responses and tolerance to nanoparticles, depending on their type, variety, and stage, as well as their genetic, physiological, and biochemical characteristics.

## Application of nanoparticles in wheat Fungal Disease management

One of the possible applications of nanoparticles in wheat fungal disease management is to use them as antifungal agents or carriers of antifungal compounds that can inhibit or kill the fungal pathogens that infect wheat. Several studies have evaluated the effectiveness of nanoparticles in controlling fungal diseases in wheat, such as Fusarium head blight, root rot, and rust, under field or experimental conditions. For example, Slavin and Bach (Fatima *et al.*, 2021) reviewed the current status and future perspectives of metal nanoparticles, such as silver, copper, zinc, and titanium, as novel antifungal agents for sustainable agriculture. They reported that metal nanoparticles have various mechanisms of action and effects on fungal pathogens and wheat plants, such as generating reactive oxygen species, interfering with fungal spore germination and hyphal growth, modulating the plant immune system, and delivering other antifungal agents. They also discussed the advantages, challenges, and future directions of nanoparticles for plant disease control.

The application of nanoparticles may also have an impact on wheat yield and quality, depending on the type, concentration, and exposure time of the nanoparticles, as well as the type, variety, and stage of the wheat plants. Some studies have shown that nanoparticles can enhance wheat growth, yield, and quality by improving nutrient uptake, photosynthesis, chlorophyll content, grain weight, and protein content. For example, (Raliya and Tarafdar, 2014) reported that zinc oxide nanoparticles increased the grain yield and protein content of wheat by 8.3 and 6.1%, respectively, compared to the control. However, some studies have also reported negative effects of nanoparticles on wheat yield and quality, such as reduced germination, biomass, and grain weight, and increased phytotoxicity and oxidative stress. For example, Wang *et al.* 2022 (Pandey *et al.*, 2023) reported that silver nanoparticles reduced the germination rate, shoot length, and root length of wheat by 16.7, 28.6, and 25.0%, respectively, compared to the control.

The application of nanoparticles in wheat fungal disease management can be compared with conventional fungicidal treatments in terms of their efficacy, cost, safety, and sustainability. Conventional fungicides are widely used in agriculture to control fungal diseases, but they have some drawbacks, such as high cost, environmental impact, resistance development, and toxicity to non-target organisms. Nanoparticles may offer some advantages over conventional fungicides, such as lower dosage, longer persistence, better penetration, controlled release, and targeted delivery of antifungal agents (Rajwade *et al.*, 2020). However, nanoparticles may also pose some challenges and limitations, such as potential toxicity and environmental impact of nanoparticles on non-target organisms, such as beneficial microbes, animals, and humans; lack of standardized methods for synthesizing, characterizing, and applying nanoparticles; and possible development of resistance or adaptation by fungal pathogens to nanoparticles. Therefore, more research is needed to optimize the synthesis, application, and evaluation of nanoparticles for controlling fungal diseases in wheat and to ensure their safety and sustainability.

## Environmental Implications

The use of nanoparticles in wheat farming may have potential environmental impacts, both positive and negative, depending on the type, concentration, and exposure of the nanoparticles, as well as the environmental conditions and interactions with other organisms. Therefore, it is important to assess the environmental implications of nanoparticle use in agricultural settings and to consider the safe and sustainable application of nanoparticles in wheat farming.

The positive environmental impacts of nanoparticle use in wheat farming may include the following:

- The reduction of runoff and nutrient leaching into the environment, due to the lower dosage, longer persistence, better penetration, controlled release, and targeted delivery of nanoparticles, compared to conventional agrochemicals (Rajwade *et al.*, 2020).
- The enhancement of soil quality and fertility, due to the improvement of soil structure, porosity, water retention, and microbial activity, by some nanoparticles, such as carbon nanotubes, graphene, and fullerenes (Yadav *et al.*, 2023).
- The mitigation of greenhouse gas emissions, due to the reduction of nitrogen fertilizer use and nitrous oxide emission, by some nanoparticles, such as zinc oxide and titanium dioxide (El-Moneim *et al.*, 2021).
- The negative environmental impacts of nanoparticle use in wheat farming may include the following:
- The toxicity and bioaccumulation of nanoparticles in non-target organisms, such as beneficial microbes, animals, and humans, due to the ingestion, inhalation, or dermal exposure of nanoparticles, which may cause oxidative stress, inflammation, DNA damage, and cell death (Rajwade *et al.*, 2020).
- The alteration of biogeochemical cycles, such as carbon, nitrogen, and phosphorus cycles, due to the interference of nanoparticles with the natural processes and functions of the soil, water, and air ecosystems (Yadav *et al.*, 2023).
- The potential transfer of nanoparticles through the food chain, from plants to animals and humans, may affect the nutritional quality and safety of the food products (El-Moneim *et al.*, 2021).

To ensure the safe and sustainable application of nanoparticles in wheat farming, some considerations and recommendations are as follows:

- The development and implementation of standardized methods and protocols for the synthesis, characterization, and application of nanoparticles, as well as the evaluation of their efficacy, toxicity, and environmental impact.
- The promotion and dissemination of the best practices and awareness of the benefits and risks of nanoparticles among the stakeholders, such as farmers, consumers, policymakers, and researchers.
- The advancement and integration of interdisciplinary and collaborative research and innovation on the application of nanoparticles in wheat farming, involving the fields of nanotechnology, agriculture, food science, environmental science, and social science (Shelar *et al*., 2023).

## Future prospects and Challenges:

Nanoparticle-based fungal disease control is a promising and emerging field that offers new opportunities and challenges for wheat agriculture. There are many future research directions and innovations that can be explored to enhance the efficacy, safety, and sustainability of nanoparticles for controlling fungal diseases in wheat. Some of these are as follows:

- The development of novel and multifunctional nanoparticles that can combine different types of antifungal agents, such as natural products, synthetic compounds, or biological agents, and deliver them in a controlled and targeted manner to the fungal pathogens, while minimizing the toxicity and environmental impact of nanoparticles on non-target organisms (Mittal *et al*., 2020).
- The design and fabrication of smart and responsive nanoparticles that can sense and respond to environmental stimuli, such as pH, temperature, light, and humidity, and modulate their antifungal activity and performance accordingly.
- The integration and application of nanorobotic systems that can accurately target and quickly destroy fungal cells, using catalytic nanoparticles, known as nanozymes, as the building blocks of the nanorobots.
- The improvement and optimization of the synthesis, characterization, and application methods and protocols of nanoparticles, as well as the evaluation of their efficacy, toxicity, and environmental impact, using standardized and reliable techniques and tools.

However, there are also some challenges and limitations associated with the widespread adoption of nanoparticle technology in wheat agriculture. Some of these are as follows:

- The potential toxicity and bioaccumulation of nanoparticles in non-target organisms, such as beneficial microbes, animals, and humans, due to the ingestion, inhalation, or dermal exposure of nanoparticles, which may cause oxidative stress, inflammation, DNA damage, and cell death.
- The alteration of biogeochemical cycles, such as carbon, nitrogen, and phosphorus cycles, due to the interference of nanoparticles with the natural processes and functions of the soil, water, and air ecosystems. The possible transfer of nanoparticles through the food chain, from plants to animals and humans, may affect the nutritional quality and safety of the food products (Xu *et al*., 2021).
- The lack of adequate regulations and guidelines for the safe handling, storage, transport, and disposal of nanoparticles, as well as the monitoring and reporting of their use and fate in the environment.
- The low public awareness and acceptance of nanoparticle technology in wheat agriculture, due to ethical, social, and economic concerns and uncertainties.

## Conclusion

In summary, the review provides an in-depth analysis of the potential of nanoparticle-based treatments for controlling fungal diseases in wheat. Key findings and insights include the diverse mechanisms of action exhibited by nanoparticles against fungal pathogens, the varying efficacy of different types of nanoparticles, the potential impact of nanoparticle application on wheat yield and quality, and the environmental implications, both positive and negative, of nanoparticle use in wheat farming.

- Recommendations for further research and practical applications in the field of nanoparticle-based fungal disease control in wheat encompass the following:
- Continued development and optimization of novel and multifunctional nanoparticles that can effectively target and control fungal diseases while minimizing adverse effects on non-target organisms.
- Rigorous assessment of the environmental implications of nanoparticle use, focusing on toxicity, bioaccumulation, and potential impacts on biogeochemical cycles and the food chain.
- Establishment and enforcement of regulations and guidelines for the safe handling, storage, transport, and disposal of nanoparticles, with a focus on monitoring and reporting their use and fate in the environment.
- Promotion of awareness among stakeholders regarding the benefits and potential risks of nanoparticle technology in wheat agriculture, taking into account ethical, social, and economic concerns.

These recommendations, when addressed through further research and practical application, can contribute to the responsible and sustainable implementation of nanoparticle-based fungal disease control in wheat, ensuring both effective disease management and environmental safety.

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**REVIEW ARTICLE**

# *In vitro* Propagation protocol for *ex-situ* Conservation of *Pyrenaria khasiana* var. *Lakhimpurensis* - An Endemic Plant to Eastern Himalaya

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## Abstract

An agile and reproducible protocol for *in vitro* propagation of *Pyrenaria khasiana* var. *lakhimpurensis*, an endemic plant to eastern Himalaya was carried out successfully. The mature seeds of this tree were collected from semi-evergreen forest of Dullung Reserve Forest, North Lakhimpur district of Assam. *In vitro* seed germination, multiple shoot induction and *in vitro* rooting experiments were carried out in woody plant medium. Benzyladenine alone and benzyladenine in combination with gibberellic acid was tried for multiple shoot induction. Benzyladenine (8 mg/L) gave highest number of shoots (2.4) per explant with a maximum shoot length of 1.45 cm observed at BA (1 mg/L). The combination of benzyladenine (1 mg/L) and gibberellic acid (4 mg/L) gave highest number of shoots (5.2) per explant with a maximum shoot length of 4.43 cm. 1 node, 2 nodes and 4 nodes were used for multiple shoot induction studies and it was observed that 4 nodes gave optimum number of shoots (40.82) and shoot length (5.039 cm) after 4 weeks of experiment. Among the auxins investigated for root induction, indole butyric acid IBA at 2 mg/L gave the highest number of roots (10.3 per shoot) with root length of 2.35 cm. Survival rate of *in vitro* raised seedlings was found to be 70% when transferred from laboratory to land. This is the first scientific report on tissue culture of the genus Pyrenaria of Theaceae family.

**Keywords:** endemic, *Pyrenaria khasiana* var. *lakhimpurensis*, *in vitro* propagation, *ex situ* conservation

## Introduction

The family Theaceae is of great economic and medicinal significance, consisting of nine genera and potentially up to 460 species. Its highest diversity is found in subtropical and tropical forests, particularly in Southeast Asia (Prince, 2007; Odyuo and Roy, 2017a). One notable genus within this family is *Pyrenaria* Blume, which includes 31 species, one subspecies, and four varieties. This genus is distributed from northeastern India and northern Myanmar to the Malaysian region, spanning southwestern China and the Indochina Peninsula (Yang *et al.*, 2000; Li *et al.*, 2011; WCSP, 2016; Tropicos, 2016). *Pyrenaria* is a less-known genus in India with a narrow range of distribution and comprises five taxa so far namely *P. barringtoniifolia* Seem. (Assam, Arunachal Pradesh and Meghalaya), *P. cherrapunjeana* Mir. (Meghalaya), *P. diospyricarpa* Kurz var. *diospyricarpa* (Assam), *P. khasiana* R. N. Paul (Meghalaya) and *P. khasiana* R. N. Paul var. *lakhimpurense* N. Odyuo and D.K. Roy (Assam) (Chauhan and Paul, 1993; Odyuo and Roy, 2017a, b; Upadhaya *et al.*, 2017; Sarma *et al.*, 2017).

*Pyrenaria khasiana* var. *lakhimpurensis* Odyuo & D. K. Roy (Fig. 1a) is endemic to the eastern Himalayas, and was described by Odyuo and Roy in 2017. This discovery was based on collections from Dullung and Kakoi

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Reserve Forest in North Lakhimpur district, Assam. It was initially reported from the southern foothills of the Himalayas in the district of North Lakhimpur, Assam, bordering Arunachal Pradesh, at an altitude of 171 meters (N27°24´36´´, E94°11´42´´) in 2011. Sarma *et al.* (2017) came across the same Theaceae species while exploring the Sonitpur District of Assam.

It is a shrub or small tree up to 5 m, barks brown or brownish grey. Young branches sparsely pubescent. Leaves oblanceolate to spathulate, 22–25 × 8.5–10.5 cm, simple, alternate, leathery, serrate, glabrous above, sparsely pubescent below, margins serrate, midrib raised below, depressed above, secondary veins 17–22, depressed above and raised below, base attenuate to cuneate, apex long acuminate; petioles sparsely pubescent in young, later glabrascent to 0.7 cm long. Flower bisexual, axillary, solitary, 4.5 cm across; pedicel silky villous, 0.3 × 0.2 cm; bracteoles 2, ovate, 0.2–0.25 × 0.25– 0.3 cm, non-foliaceous, leathery, silky villous outside, glabrous inside, apex obtuse Sepals persistent, 7, ovate, orbicular to obovate, 0.5–1.5 × 0.7–1.2 cm, non-foliaceous, silky villous outside, glabrous inside. Petals 7, white, unequal, oblanceolate–obovate, 1.0–2.0 × 0.5–1.5 cm, silky villous outside, glabrous inside, apex rounded. Stamens numerous, basally connate; filaments glabrous, *c.* 1 cm long, outer whorl slightly adnate to petals; anther dorsifixed, bilobed. Ovary superior, globsose, 5-loculed, 0.3–0.4 cm long, axile placentation; style 5, free, *c.* 0.7 cm long. Fruit subglobose, 2.5–3.5 × 2.3–3.2 cm, glabrous, apex crowned with 5 stigmatic protuberances, with persistent calyx at base, 5-loculed, fleshy; pericarp yellowish, *c.* 0.4–0.6 cm thick, fleshy when fresh but becoming spongy when dry. Seeds 5–7, 0.8–1 cm, laterally compressed hemispheric, occasionally 2 per locule; testa bony. Flowering & Fruiting: February – August. This species is closely related to *P. khasiana* R.N. Paul but differs from it by pedicel silky villous (vs glabrous), bracteoles non-foliaceous (vs foliaceous), sepals ovate, orbicular to obovate, non-foliaceous (vs suborbicular, foliaceous) and petals oblanceolate-obovate (vs obovate to suborbicular).

Reviewing the protologue data and considering the current population, density, distribution area, and other factors in consultation with the IUCN categorization guidelines, this taxon can be classified as Critically Endangered- B1B2 (a, b); C2a (i); D category (Sarma *et al.*, 2017). Given that this species falls under the Critically Endangered category, its conservation is of utmost importance. To conserve this threatened Theaceous variety, both *ex situ* and *in situ* methods should be employed.

Species conservation primarily relies on the management of natural populations and their native habitats, known as *in situ* conservation. However, *ex situ* techniques can play a complementary role in conservation efforts (Maunder *et al.*, 1998; Ramsay *et al.*, 2000). In particular, *in vitro* propagation is a highly effective method for rapidly cultivating endangered plant species that have limited reproductive capabilities and are found in threatened environments (Fay, 1994). Plant tissue culture is an important propagation technique to reproduce plants on a superior scale in a short time (Singh, 2018). Micropropagation, which offers several advantages over traditional vegetative propagation, holds significant promise for supporting conservation initiatives and harnessing genetic diversity (Azad *et al.*, 2022). This technique is of particular importance for rare and endangered endemic plant species, as it enables the establishment of cultures from minimal initial plant material for subsequent multiplication (Benson *et al.*, 2000).

Till date, there have been no reports on *in vitro* propagation of *Pyrenaria* species. Consequently, the central aim of this research is to pioneer the development of a highly effective *in vitro* protocol for the mass multiplication of this species. This endeavour is poised to make a substantial contribution to the preservation of this distinctive plant species within its native habitat. Remarkably, this study represents the inaugural effort to establish a successful micropropagation technique for the conservation of this endemic species native to the eastern Himalayas.

## Materials and methods

### Collection of explants

The mature fruits (Fig. 1b) were collected from Dullung Reserve Forest, North Lakhimpur district of Assam.

### *In vitro* seed germination

Woody Plant Medium (WPM, Lloyd and McCown 1981) supplemented with 3% (*w/v*) sucrose and 0.8% agar (*w/v*) was used for *in vitro* seed germination studies. pH of the medium was adjusted to 5.8. About 50ml of the medium was dispended in 250 ml conical flask (Borosil Glass Works Ltd., Mumbai, India). Seeds (Fig. 1c) were disinfected with 10% (*v/v*) sodium hypochlorite solution (4% w/v solution, HiMedia Laboratories Pvt. Ltd.) with 2-3 drops of Tween®-80 liquid detergent per 100ml for 15-20 minutes, subsequently washed 4-5 times in sterile distilled water under laminar air flow chamber. Five seeds per 250ml conical flask were inoculated and all cultures were maintained in culture room under warm white fluorescent light at a 16h photoperiod and at 25±2°C. Initiation of germination was recorded after two weeks of culture.

### Multiple shoot induction

Nodal segments from 2 months old aseptic seedlings were cultured on WPM containing various concentrations of 6-Benzylaminopurine (6-BAP) (0.5, 1, 2, 4, 8 mg/L) alone and in combination with Gibberellic acid ($GA_3$) (0.5, 1, 2, 4, 8 mg/L) were used for multiple shoot induction. pH was adjusted to 5.8 and gelled with 0.8% agar (*w/v*) before autoclaving at 121°C for 20 minutes at 1.05 kg/cm$^2$ pressure. Basal WPM was used as control and the experiments were repeated thrice with ten replicates per treatment. 1 node, 2 nodes and 4 nodes explants were also tested for better shoot proliferation. Cultures were maintained at 25±2ºC temperature under 16/8 hr (light/ dark) photoperiod under white fluorescent light. Ten replicates were set up per experiment for comparing the effect of different concentrations of growth regulators and the experiments were repeated thrice. Observation for shoot initiation and number of shoots produced were recorded after 3 weeks and up to 12 weeks of culture.

### Rooting

*In vitro* raised shoots of approximately 5 to 6 cm length isolated from healthy shoot clump were used for *in vitro* rooting. WPM gelled with 0.8% agar supplemented with different concentrations of auxins *viz*., á-Naphthalene acetic acid (NAA), Indole butyric acid (IBA) and Indole-3-acetic acid (IAA) were tested for root induction. Data for root initiation, number of roots and the root length were recorded after 8 weeks of culture. The experiment was repeated thrice with 10 replicates per treatment.

### Data Analysis

Data analysis was conducted using OriginPro 8SRO v8.0725 (B725) software developed by OriginLab® Corporation based in Northampton, MA. To compare the means of different treatments, a one-way ANOVA (Analysis of Variance) was performed. Tukey's test was then used to determine significant differences between treatment means at a significance level of P = 0.05

## Acclimatization

Healthy *in vitro* grown plants with well-developed roots (Fig. 1j) were carefully removed from the conical flask to avoid any damage to the plant. They were then washed with tap water to remove any residual media clinging to the roots. To minimize microbial infection, the plants were dipped in 0.1% Bavistin (w/v) solution for one hour. After the treatment, the plants were rinsed with tap water and transferred to polythene bags filled with a mixture of garden soil and leaf mould in a 1:1 ratio and kept in polyhouse (i.e., 25°C, RH 60%). The plants were watered on every alternate day and survival percentage was noted.

## Results and discussion

### *In vitro* seed germination

*In vitro* seed germination was found to be remarkably efficient on basal WPM (Fig. 1d), with the majority of seeds demonstrating signs of germination within a short time frame of just 2 weeks. This rapid response to the basal WPM indicates its suitability as a nutrient-rich substrate for initiating seed germination in *Pyrenaria khasiana* var. *lakhimpurensis*. However, an intriguing observation was the cessation of seed germination beyond the 8th week of culture, which suggests a possible limitation or shift in the physiological state of the seeds as they progress in culture.

**Table 1. Effect of different concentrations of 6-BAP alone and in combination with GA3 in multiple shoot induction**

| BA (mg/L) | GA$_3$ (mg/L) | 3rd Week | | 6th Week | | 9th Week | | 12th Week | |
|---|---|---|---|---|---|---|---|---|---|
| | | No. of shoots | Shoot length (cm) | No. of shoots | Shoot length (cm) | No. of shoots | Shoot length (cm) | No. of shoots | Shoot length (cm) |
| 0 | 0 | 0.6$^{de}$ | 0.19$^{gh}$ | 0.7$^{fg}$ | 0.26$^{ef}$ | 0.75$^{e}$ | 0.34$^{g}$ | 0.7$^{g}$ | 0.48$^{g}$ |
| 0.5 | - | 1.1$^{cd}$ | 0.25$^{gh}$ | 2.1$^{ab}$ | 0.56$^{ef}$ | 2.1$^{bcd}$ | 0.72$^{efg}$ | 2.3$^{cdef}$ | 0.83$^{efg}$ |
| 1 | - | 1.4$^{bc}$ | 0.36$^{fgh}$ | 1.8$^{abcd}$ | 0.82$^{def}$ | 1.8b$^{cde}$ | 1.21$^{defg}$ | 2.2$^{cdefg}$ | 1.45$^{cdefg}$ |
| 2 | - | 0.9$^{cd}$ | 0.22$^{gh}$ | 1.6$^{bcde}$ | 0.6$^{ef}$ | 1.5b$^{cde}$ | 0.8e$^{fg}$ | 1.8$^{defg}$ | 1.09d$^{efg}$ |
| 4 | - | 0.7$^{d}$ | 0.19$^{gh}$ | 1.7$^{abcd}$ | 0.38$^{ef}$ | 1.7$^{bcde}$ | 0.62$^{fg}$ | 2$^{cdefg}$ | 0.77$^{fg}$ |
| 8 | - | 0.9$^{cd}$ | 0.24$^{gh}$ | 1.7$^{abcd}$ | 0.25$^{ef}$ | 1.9$^{bcde}$ | 0.37$^{g}$ | 2.4$^{cde}$ | 0.81$^{efg}$ |
| 0.5 | 0.5 | 0.9$^{cd}$ | 1.37$^{ab}$ | 0.8$^{efg}$ | 1.95$^{a}$ | 1$^{de}$ | 2.41$^{abcd}$ | 1$^{efg}$ | 2.85$^{bcd}$ |
| 0.5 | 1 | 0.7$^{d}$ | 0.53$^{efg}$ | 0.7$^{fg}$ | 0.71$^{ef}$ | 0.7$^{e}$ | 1.44$^{cdefg}$ | 0.8$^{fg}$ | 2.41$^{bcdef}$ |
| 0.5 | 2 | 1.1$^{cd}$ | 1.24$^{abc}$ | 1.2$^{cdef}$ | 1.6$^{abcd}$ | 1.9$^{bcde}$ | 2.39$^{abcd}$ | 2.5$^{cde}$ | 2.46$^{bcde}$ |
| 0.5 | 4 | 1$^{cd}$ | 0.92$^{bcde}$ | 1.3$^{bcdef}$ | 0.89$^{cde}$ | 1.8$^{bcde}$ | 1.77$^{bcdef}$ | 1.9$^{defg}$ | 2.35$^{bcdef}$ |
| 0.5 | 8 | 0.8$^{cd}$ | 0.66$^{defg}$ | 1$^{def}$ | 0.97$^{cde}$ | 1.7$^{bcde}$ | 1.73$^{bcdefg}$ | 2.7$^{bcd}$ | 2.03$^{cdefg}$ |
| 1 | 0.5 | 1.4$^{bc}$ | 0.98$^{bcde}$ | 1.7$^{abcd}$ | 0.89$^{cde}$ | 2.5$^{abc}$ | 1.64$^{bcdefg}$ | 3.5$^{bc}$ | 1.78$^{cdefg}$ |
| 1 | 1 | 1.8$^{ab}$ | 1.55$^{a}$ | 2$^{abc}$ | 1.86$^{ab}$ | 2.1$^{bcd}$ | 2.9$^{ab}$ | 2.7$^{bcd}$ | 3.96$^{ab}$ |
| 1 | 2 | 1.4$^{bc}$ | 1.14$^{abcd}$ | 2.1$^{ab}$ | 1.76$^{abc}$ | 2.7$^{ab}$ | 2.83$^{abc}$ | 4.1$^{ab}$ | 3.96$^{ab}$ |
| 1 | 4 | 2.3$^{a}$ | 1.54$^{a}$ | 2.5$^{a}$ | 2.12$^{a}$ | 3.7$^{a}$ | 3.75$^{a}$ | 5.2$^{a}$ | 4.43$^{a}$ |
| 1 | 8 | 0.6$^{de}$ | 0.78$^{cdef}$ | 0.6$^{fg}$ | 1.02$^{bcde}$ | 0.8$^{e}$ | 1.2$^{defg}$ | 1$^{efg}$ | 1.49$^{cdefg}$ |
| 2 | 0.5 | 0$^{e}$ | 0$^{h}$ | 0$^{g}$ | 0$^{g}$ | 1.9$^{bcde}$ | 0.89$^{efg}$ | 1.9$^{defg}$ | 1$^{defg}$ |
| 2 | 1 | 0$^{e}$ | 0$^{h}$ | 0$^{g}$ | 0$^{g}$ | 1.6$^{bcde}$ | 1.03$^{defg}$ | 1.6$^{defg}$ | 1.09$^{defg}$ |
| 2 | 2 | 0$^{e}$ | 0$^{h}$ | 0$^{g}$ | 0$^{g}$ | 1.9$^{bcde}$ | 1.12$^{defg}$ | 2.2$^{cdefg}$ | 1.32$^{cdefg}$ |
| 2 | 4 | 0$^{e}$ | 0$^{h}$ | 0$^{g}$ | 0$^{g}$ | 1.4$^{cde}$ | 1.34$^{defg}$ | 1.8$^{defg}$ | 2.21$^{cdef}$ |

Data was statistically analyzed using one-way ANOVA and Tukey's test (P = 0.05) in Origin Pro 8SRO v8.0725 (B725). Each value is the average of three replications (n = 10). Different letters following numbers in the same column indicate significant differences, while means with the same letters are not significantly different.

### Multiple shoot induction

### Effect of 6-BAP and $GA_3$ on shoot multiplication

In this study, the combination of 6-BAP at 1 mg/L and $GA_3$ at 4 mg/L proved to be the most effective treatment for promoting multiple shoot from nodal explants (Fig. 1e). This combination resulted in the highest number of shoots per explant, with a maximum of 5.2 shoots observed after 12 weeks of culture. Moreover, these shoots achieved a maximum length of 4.43 cm, indicating their remarkable ability to enhance both shoot proliferation and elongation (Table 1). These results underscore the synergistic effects of 6-BAP and $GA_3$ on shoot organogenesis.

**Fig. 1.** (a) *Pyrenaria khasiana* var. *Lakhimpurensis* mother plant in its natural habitat (b) Mature fruits (c) Seeds inoculated for *in vitro* germination (d) *in vitro* seed germination on Woody Plant Medium (WPM) (e) Multiple shoot initiation on WPM with 6-BAP at 1 mg/L and $GA_3$ at 4 mg/L after 4 weeks in culture (f) Multiple shoots developed using 1 node (g) Multiple shoots developed using 2 nodes (h) Multiple shoots developed using 4 nodes (i) *in vitro* rooting after 12 weeks in culture on WPM containing 2 mg/L IBA (j) *in vitro* rooted plants ready for acclimatization (k) Six months old healthy plants grown in polythene bags for acclimatization (l) Established plant in office garden of BSI, Shillong

Cytokinins like 6-BAP are typically employed to stimulate growth and development, particularly promoting cell division. At higher concentrations, they can induce adventitious shoot formation while inhibiting root formation (Widhianata, 2019). Additionally, the presence of $GA_3$ played a crucial role in inducing shoot elongation, resulting in prominent nodal segments that could be used for further multiplication during subculture (Gonbad, 2014). On the other hand, gibberellins, such as $GA_3$, generally induce internode elongation and growth of plants or buds *in vitro*, and they are known to break dormancy in isolated embryos or seeds. Gibberellins tend to inhibit the formation of adventitious roots. (Gana, 2010)

Furthermore, it was observed that 6-BAP alone produced only two-three shoots and higher concentrations of 6-BAP resulted in a reduction in the number of shoots, necrosis, and shoot fasciation. This reduction in shoot numbers may be attributed to the inhibition of adventitious meristem elongation, possibly due to the use of higher 6-BAP concentration (Borchetia *et al*., 2009).

**Effect of node number on shoot multiplication**

Node culture is a tissue culture method in which axillary shoot buds from shoots taken during active or resting period are transferred to artificial nutrient medium and developed into plants. A complete plant can be obtained from different organs of the plant such as buds, leaves, shoots and roots. These organs can be reproduced in a short time when they are cultured in artificial media under sterile conditions. A large number of plants are produced using micropropagation (Gulzar *et al*., 2020).

The results of the multiple shoot induction experiment conducted over a 4-month period with different numbers of nodal explants revealed interesting results (Table 2). It is evident from the data that when using a single node explant, both the number of shoots and their length gradually increased from the first to the fourth month of culture. This observation implies that a single node explant can effectively generate multiple shoots over time, reaching its peak with 7.82 shoots in the fourth month, along with a significant increase in shoot length, which extended to 4.51 cm (Fig. 1f). These results underscore the impact of the number of nodal explants on multiple shoot induction, influenced by the varying physiological and developmental characteristics of these explants. The gradual increase in shoot numbers and length observed with single node explants over the 4-month period suggests that, although slower to initiate, they can serve as a sustained and reliable source of multiple shoots. This finding aligns with Chen *et al.* 2020 that have advocated for the utilization of single-node explants in long-term micropropagation protocols.

In contrast, when using two nodes or four nodes, the number of shoots and shoot length increased substantially. Explants with two nodes exhibited a remarkable surge in shoot numbers, with 24.3 shoots produced in the fourth month, and a steady increase in shoot length (5.66 cm) across the months (Fig. 1g). Similarly, explants with four nodes displayed an even more pronounced increase in shoot numbers, attaining 40.82 shoots in the fourth month. Although the shoot length fluctuated slightly, it remained relatively high (5.039 cm) (Fig. 1h). These findings indicate that explants with two or four nodes result in a rapid and robust response in terms of multiple shoot induction. On the other hand, explants with two or four nodes exhibited a more rapid and vigorous response in the early months of culture, producing a larger number of shoots. This may be associated with the presence of more meristematic regions and an increased potential for axillary bud outgrowth in multi-node explants.

These results corroborate with the findings of Buckseth *et al.*, (2017) and Venkatasalam *et al.*, (2012) where they emphasize the rapidity of response associated with multi-node explants.

**Table 2. Response of different nodal explants on shoot number and shoot length in 6-BAP at 1 mg/L and GA3 at 4 mg/L combination**

| No. of nodal explants | 1 month | | 2 months | | 3 months | | 4 months | |
|---|---|---|---|---|---|---|---|---|
| | No. of shoots | Shoot length (cm) | No. of shoots | Shoot length (cm) | No. of shoots | Shoot length (cm) | No. of shoots | Shoot length (cm) |
| 1 node | 2.39$^{c}$ | 3.38$^{a}$ | 4.47$^{c}$ | 3.98$^{a}$ | 5$^{c}$ | 4.32$^{a}$ | 7.82$^{c}$ | 4.51$^{a}$ |
| 2 nodes | 11.04b | 3.45$^{a}$ | 15.95$^{b}$ | 4.65$^{a}$ | 21.21$^{b}$ | 4.91$^{a}$ | 24.30$^{b}$ | 5.66$^{a}$ |
| 4 nodes | 17.34$^{a}$ | 3.8$^{a}$ | 22.69$^{a}$ | 4.65$^{a}$ | 35.21$^{a}$ | 4.25$^{a}$ | 40.82$^{a}$ | 5.039$^{a}$ |

**Table 3. Effect of different auxins on root induction after 8 weaks in culture**

| PGR | Concentration (mg/L) | Mean no. of roots | Mean root length (cm) |
|---|---|---|---|
| control | - | 3.4$^{c}$ | 0.96$^{ef}$ |
| IBA | 0.5 | 2.3$^{c}$ | 0.85$^{f}$ |
| IBA | 1 | 9.2$^{ab}$ | 1.73$^{bcd}$ |
| IBA | 2 | 10.3$^{a}$ | 2.35$^{b}$ |
| IBA | 4 | 7.5$^{ab}$ | 1.53$^{cdef}$ |
| IBA | 8 | 7$^{b}$ | 1.92$^{bc}$ |
| IAA | 0.5 | 2.5$^{c}$ | 1.125$^{def}$ |
| IAA | 1 | 2.65$^{c}$ | 0.97$^{ef}$ |
| IAA | 2 | 2.05$^{c}$ | 0.865$^{f}$ |
| IAA | 4 | 3.15$^{c}$ | 1.2d$^{ef}$ |
| IAA | 8 | 2.5$^{c}$ | 1.04$^{def}$ |
| NAA | 0.5 | 2.6$^{c}$ | 1.64$^{bcde}$ |
| NAA | 1 | 2$^{c}$ | 1.6$^{cde}$ |
| NAA | 2 | 2$^{c}$ | 1.32$^{cdef}$ |
| NAA | 4 | 1.8$^{c}$ | 1.14$^{def}$ |
| NAA | 8 | 1.6$^{c}$ | 1.26$^{cdef}$ |

Data was statistically analyzed using one-way ANOVA and Tukey's test (P = 0.05) in Origin Pro 8SRO v8.0725 (B725). Each value is the average of three replications (n = 10). Different letters following numbers in the same column indicate significant differences, while means with the same letters are not significantly different.

## Rooting

Among the auxins tested, IBA exhibited a dose-dependent effect on root development (Table 3). At lower concentrations (0.5 mg/L), IBA led to 2.3 roots with a root length of 0.85 cm, while at 1 mg/L, the number of roots increased to 9.2, and root length extended to 1.73 cm. The highest IBA concentration (2 mg/L) yielded the most promising results, with an average of 10.3 roots and a significantly enhanced mean root length of 2.35 cm (Fig. 1i). Similar results were reported in *Camellia japonica* L. (Wojtania *et al*., 2011) where the best rooting response was observed in IBA. At higher IBA concentrations (4 mg/L and 8 mg/L), the number of roots and root length remained relatively stable, though slightly lower than the optimal 2 mg/L concentration.

In case of IAA and NAA, the effect on root development was less pronounced. Regardless of the concentration, IAA and NAA treatments resulted in only a limited number of roots, with IAA producing root lengths ranging from 0.865 cm to 1.125 cm and NAA inducing root lengths between 1.14 cm and 1.64 cm. Rooting was also observed in hormone-free WPM. But it was observed that the number of roots was less and root length was not comparable with hormone based medium results. This suggests that the presence of endogenous salicylic acid, an important compound for plant development and growth, including *in vitro* rooting, is the factor that causes adventitious roots to form on auxin-free basal media (Khan *et al*., 2011).

Data were statistically analyzed using one-way ANOVA and Tukey's test (P = 0.05) in Origin Pro 8SRO v8.0725 (B725). Each value is the average of three replications (n = 10). Different letters following numbers in the same column indicate significant differences, while means with the same letters are not significantly different.

## Acclimatization and reintroduction

Approximately 70% (300 Nos.) of *in vitro* raised plantlets, which had developed roots, successfully adapted to greenhouse after twelve weeks of transplanting and were found to be growing well (Figure 1k). No abnormalities were observed and regenerated plants were morphologically similar to the mother plant. Around 60 acclimatized plants were introduced to enhance the greenery at BSI, ERC office garden in Shillong and the Experimental Botanic Garden in Barapani, Shillong (Fig. 1l). Approximately 100 plants have been distributed to various organizations, institutions and stakeholders in Meghalaya for reintroduction. The remaining acclimatized plants have been preserved, with the intention of facilitating future reintroductions and distributing them to botanical gardens that share similar climatic conditions, thus contributing to their *ex situ* conservation efforts.

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**RESEARCH ARTICLE**

# *Chlorella vulgaris:* Physiological Attributes and Characterization

**Abhinav Singh[1], Baby Verma[2]*, Yashi Rathi[3], Neelesh Kapoor[4] and Rakesh Singh Sengar[5]**

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## Abstract

Oleaginous microalgae *Chlorella vulgaris* has been identified as potential algal species for biodiesel production. The information on behaviour of this alga under abiotic stress (pH, ambient temperature and photoperiod) and nutrient stress is limited. This study aimed to investigate the effect of pH (2, 5, 7, 9 and 11), temperature (10, 20, 30 and 40 °C), photoperiod (continuous light, 24:0 h; light and dark, 18:6 h; and continuous dark, 0:24 h) and nutrient load (N–$NO_3$- and $PO_4^{-3}$) on growth and chemical composition of harvested biomass of *Chlorella*. Result indicates the optimum ranges of abiotic conditions as 18:6 h light/dark regimes (photoperiod), 7–9 pH and 20–30 °C temperature for algal cultivations.

**Keywords:** *Chlorella vulgaris*, micnoalgae, chlorophyll, charactenzation, green algae

## Introduction

Microalgae are mainly considered as a source for production of energy (biofuel), Poly-Unsaturated Fatty Acids (PUFA) or pigments but they also contain high value molecules such as proteins and polysaccharides that are often ignored during energy or pigments recovery (Pignolet *et al.*,2013). Plant biomass is a form of energy which is located in different plant parts such as leaf, stem and root. In extent, research on enhancement of biomass production is a continuous process in plant science or agricultural sector to provide food, medicine, nutraceuticals etc., for the future generations (Khoo *et al.*, 2011 and Welfle *et al.*, 2014). Moreover, estimation of yield through biomass content is very common process and most of the researchers will use this method to get appropriated data all over the world. Evaluation of content of chlorophyll such as chlorophyll a and chlorophyll b, in turn total chlorophylls and carotenoids, namely xanthophyll and carotene is one of the best ways to estimate the biomass of particular organism. Chlorophylls (Chl. a and Chl. b) are abundant in nature which gives green color to plants and have light harvesting role in the process of photosynthesis (Costache *et al.*, 2012). In addition these are also used as antioxidant and anti-mutagenic agents and helpful in pharamaceutical and cosmetic industries (Jayashree *et al.*, 2016). Carotenoids contain yellow, orange and red colored xanthophylls (x) and carotenes (c) which are distributed widely in green, red, blue-green, red and brown algae (Khoo *et al.*, 2011).

Large-scale microalgal production is a well-established industry (Borowitzka, 1999) with microalgae being used for human nutrition, animal, aquaculture feed, biofertilizers and valuable chemicals such as carotenoids, phycobilins, fatty acids and other secondary metabolites with pharmaceutical applications (Borowitzka 1992; Chisti, 2006). Non-pigment proteins from microalgae are not or poorly valorised except for some, used in feed and as food (Spolaore *et al.*, 2006; Becker,

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2007) the functional properties (water and fat adsorption capacity, emulsification capacity) of the entire biomass of some microalgae were reported by Guil-Guerrero *et al.* (2004). Cell lysis must be achieved before extraction of the protein fraction. The most important cell lysis technique use mechanical action (high pressure homogenisers, bead mills), ultrasounds, enzymatic or chemical treatments, thermal or osmotic shocks (repeated freezing/thawing) (Middelberg, 1995). Microalgae produce different valuable components, such as carbohydrates, long chain fatty acids, pigments and proteins. Carbohydrate fraction consists mostly of cellulose and starch without any lignin residue, so it can be easily used for the fermentation process (John *et al.*, 2011). Lipids can be used as a source for biofuels, as building blocks in chemical industry, and edible oils for the food and health market. Application of bio-refinery concept (exploiting all products contained in biomass) can further enhance microalgal cultivation feasibility (Di Caprio *et al.*, 2015b). Downstream operations should be mild, inexpensive and low energy consuming, avoiding product damages and enforcing process economy (Vanthoor-Koopmans *et al.*, 2012). Solvent extraction is one of the most commonly employed methods in lipid extraction for biodiesel production. (Singh and Gu, 2010; Halim *et al.*, 2012; Grima *et al.*, 2013). Microalgal cell walls are complex and poorly understood (Popper *et al.*, 2010).The *Chlorella* intraspecies variation in cell walls as well as variations observed in a single strain grown under different conditions can be dramatic and thus it is difficult to predict which of the compounds noted below will be present in any one strain. While some *Chlorella* species have only a single microfibrillar layer, others have two layers with the microfibrillar layer proximal to the cytoplasmic membrane and a mono or trilaminar outer layer (Yamada *et al.*, 1982).

Microalgae produce carbohydrates for two main purposes: they serve as a structural component in the cell wall and as a component for intracellular storage. As a storage compound, carbohydrates provide the energy needed for the metabolism of the microalgae and allow them to survive temporarily in the dark when needed (Khan *et al.*, 2016). Microalgae components (such as proteins, lipids, and carbohydrates) allow them to adapt to changing environmental conditions for their growth (Markou and Muelaert, 2016).

**Fig.1:** Photomicrograph of green algae *Chlorella*

## Material and Methods

**Organisms:** Two Algal strains (CCC125 and CCC129) from genus *Chlorella* were procured from Algal Culture Collection unit of the Department of Microbiology, Ch. Charan Singh University, Meerut, U.P., India.

### Growth and Maintenance

Algal strains (*Chlorella*) grown and maintained in chemically defined, modified BG-11 Medium (Stanier,1971) at 28 ± 2°C under a light intensity of 52-55 μmol photon $m^{-2}$ $s^{-1}$ and L:D cycles of 16:8 hours. pH of the medium was maintained in the range of 7.5 for optimal growth of cultures. Solid agar based medium was prepared by dissolving 12-15 g of purified agar in 1 liter of medium (1.2-1.5%) and autoclaved before use for maintenance of the cultures. Mass production of strains on large scale was done in photo bioreactor (Applikon Inc.) and raceways system. After 14 days of incubation, the cultures were subjected to streaking on agar based BG-11 Medium for obtaining discrete colonies under similar cultural conditions. These colonies were picked up and inoculated in 50 mL flasks containing 20 mL BG-11 Medium and incubated till exponential phase, till 14th day. The strains used in the present study varied in their morphological characters and the identification was authenticated based upon the keys given by Geitler (1932) and Desikachary (1959). The purity of the cultures was examined by microscopic observations at different stages of growth and the purity was maintained by streaking the cultures regularly on agar plates having suitable media.

**Fig.2:** Maintenance of algal culture in culture room in Haffkine flask.

## Purification and axenization

In order to obtain axenic strains of *Chlorella* cultures, the method involving triple antibiotic solution was employed (Kaushik, 1987).

### Estimation of pigments

Pigments were estimated at different stages of incubation (7th, 14th, 21st and 28th day) using standard protocols.

### Chlorophyll

As described by McKinney, 1941, a known volume (10 ml) of homogenized Algal suspension was taken and subjected to centrifugation (4000 g, 10 min.). The chlorophyll was extracted from pellet with similar volume of methanol (95%) in a water bath (60 °C, 30 min.). The suspension was centrifuged and the absorbance of the supernatant was read at 650 nm and 665 nm against methanol (95%) as blank. The concentration of chlorophyll was calculated using the formula:

Total chlorophyll (mg/ml) = $2.55 \times 10^{2} E_{650} + 0.4 \times 10^{2} E_{665}$

Where, $E_{650}$ = Absorbance at 650 nm, $E_{665}$ = Absorbance at 665 nm.

Carotenoids (mg/mL) = $[D \times V \times f \, x10] / 2500$

Where, D = Absorbance at 450 nm; V = Volume of the extract and f = Dilution factor.

It is assumed that these pigments have an average extinction coefficient of 2500.

### Total Soluble Proteins

A known volume (0.5 ml) of homogenized algal suspension at different days of incubation (7th, 14th, 21th, 28thday) was taken in a test tube. To this, 0.5 ml of sodium hydroxide (1N) solution was added. The tubes were then heated in a boiling water bath for 10 min. and cooled in running tap water. Subsequently, 2.5 ml of reagent (Folin-ciocalteau reagent, 1N with 5% sodium carbonate and 0.5% copper sulphate penta hydrate solution in1% sodium potassium tartarate) was added in each test-tube and the tubes were kept at room temperature for 10 min. The intensity of blue color was read as absorbance at 650 nm against appropriate blank. The protein content was estimated using a standard calibration curve prepared from bovine serum albumin and expressed in terms of mg/mL.

## Results and Discussion

The final results for different physiological parameters at different days of incubation (7, 14, 21 and 28 Days) revealed the following:

1. For chlorophyll content, the *Chlorella vulgaris* strain CCC 129 showed maximum concentration at 14th days of incubation.

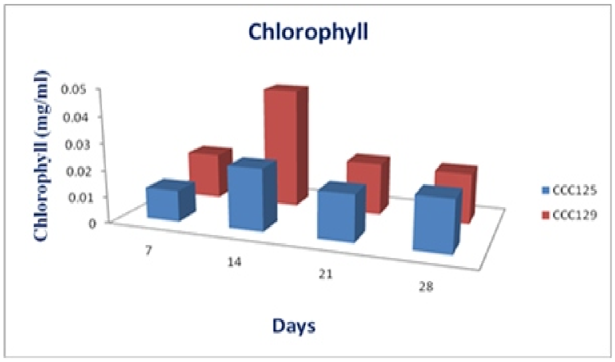

**Fig.3: Chlorophyll (mg/ml) at different days (7, 14, 21 and 28) of incubations**

2. For carotenoids, strain CCC 129 displayed the maximum concentration at 14th day of incubation.

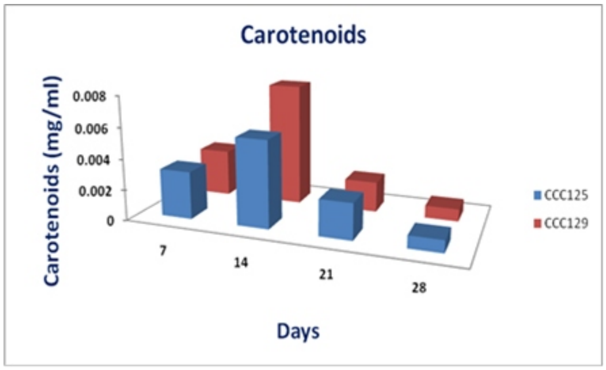

Fig.4: Chlorophyll (mg/ml) at different days (7, 14, 21 and 28) of incubations

3. For Total Soluble Proteins, the strain CCC 129 showed maximum concentration at 14th days of incubation.

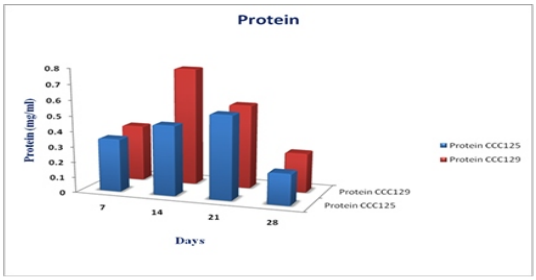

**Fig.5: Protein in mg/ml at different days (7,14,21 and 28) of incubation**

4. For the Dry Weight, strain CCC 125 yielded the maximum dry weight at 14th days of incubation

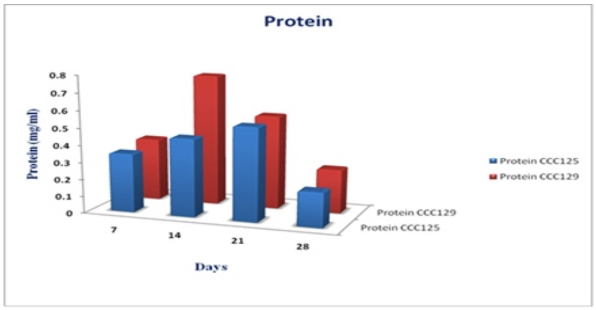

**Fig.5: Protein in mg/ml at different days (7,14,21 and 28) of incubation**

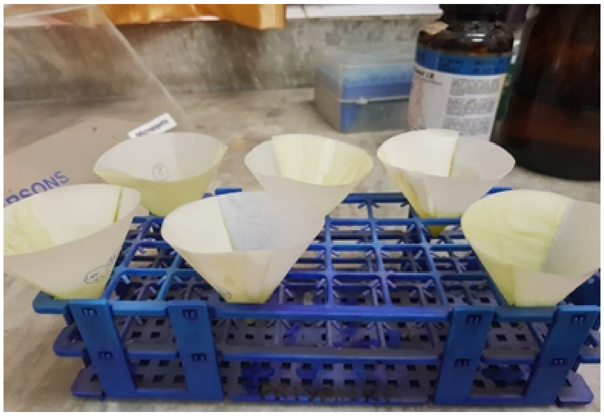

**Fig.7: Dry weight analysis**

## Conclusion

The main conclusions of this research work are that the Chlorophyll, Carotenoids and Total soluble proteins were maximum in culture of *Chlorella vulgaris* strain CCC 129 at 14 days. Whereas, Dry weight was found to be maximum in culture of *Chlorella vulgaris* strain CCC 125. So it can be well advocated from this study that the strain CCC129 of *Chlorella vulgaris* can be used for further mass production, because it has higher nutraceutical values.

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**REVIEW ARTICLE**

# Liquid Biopsy: Illuminating Cancer Diagnosis with New Horizons

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## Abstract

The landscape of cancer diagnosis is undergoing a paradigm shift with the advent of liquid biopsy, a non-invasive approach that analyzes circulating biomarkers in bodily fluids. This review illuminates the transformative potential of liquid biopsy in revolutionizing cancer diagnostics, offering a comprehensive overview of its principles, technologies and clinical applications. The metaphorical "illuminating" encapsulates the hope and brightness liquid biopsy brings to the field. The paper begins by highlighting the limitations of traditional tissue biopsies, emphasizing the need for a more dynamic and less invasive methodology. The principles of liquid biopsy, centered on circulating tumour DNA (ctDNA), circulating tumour cells (CTCs) and other cancer-associated components, are explored. Various technologies, including next-generation sequencing (NGS) and digital polymerase chain reaction (dPCR), are discussed for their roles in liquid biopsy analysis. The clinical applications of liquid biopsy are examined, showcasing its efficacy in early detection, treatment selection, monitoring treatment response and detecting minimal residual disease. Challenges such as sensitivity, specificity and standardization are critically addressed and the paper concludes with a comparative analysis of liquid biopsy against traditional methods, highlighting its potential to reshape the landscape of cancer diagnostics. This review serves as a valuable resource for researchers, clinicians and policymakers navigating the forefront of cancer diagnostics.

**Keywords:** Liquid biopsy, Circulating tumour DNA (ctDNA), Cancer diagnostics, Non-invasive testing, Biomarkers, Next-generation sequencing (NGS), Minimal residual disease, Precision medicine, Early cancer detection, Genomic profiling, Personalized therapy

## Introduction

Cancer is a complex and multifaceted group of diseases that continues to pose a formidable challenge to global healthcare systems. The conventional methods for diagnosting and monitoring cancer, often reliant on invasive tissue biopsies, face limitations such as sampling bias, patient discomfort and the inability to capture the dynamic nature of the disease. In this context, the advent of liquid biopsy has ushered in a new era in cancer diagnostics, promising a non-invasive, sensitive and comprehensive approach to detecting molecular alterations associated with malignancies. This review explores the transformative landscape of liquid biopsy, illuminating the path to enhanced cancer diagnosis and management.

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Traditionally, the gold standard for cancer diagnosis has been tissue biopsy, which involves the surgical removal of a tissue sample for pathological analysis. However, this approach has significant drawbacks, including the potential for sampling errors due to tumour heterogeneity and the inability to perform serial sampling to monitor disease progression or treatment response (Hanahan and Weinberg, 2011). Recognizing these limitations, researchers and clinicians have turned to liquid biopsy, a methodology that involves the analysis of various biomarkers present in bodily fluids, primarily blood.

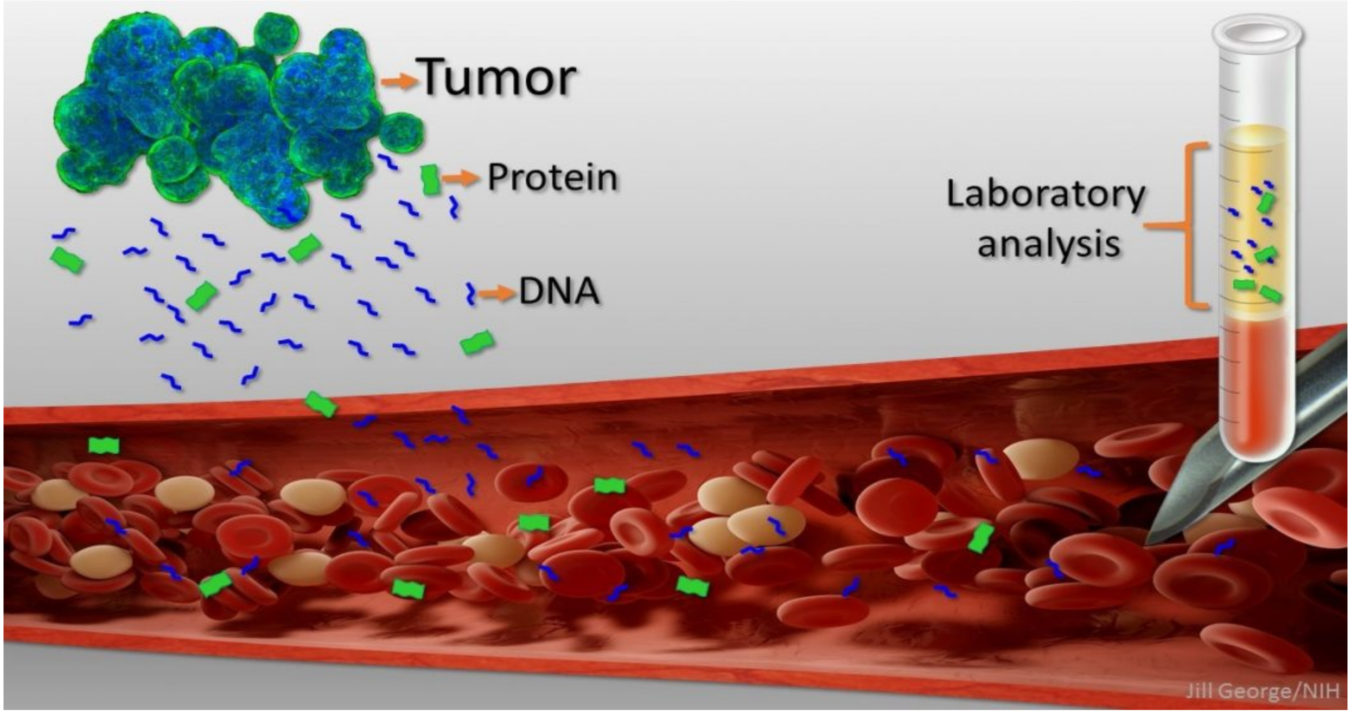

**Fig. 1**: Diagramatic illustration of liquid biopsy

The cornerstone of liquid biopsy is the identification and analysis of Circulating Tumour DNA (ctDNA), Circulating Tumour Cells (CTCs) and other cancer-associated components present in the bloodstream. ctDNA, in particular, represents fragmented DNA shed by tumour cells into circulation, carrying genetic alterations reflective of the tumour's genomic landscape (Siravegna *et al.*, 2017). This approach provides a dynamic and real-time snapshot of the tumour's molecular profile, allowing for early detection, monitoring of treatment response and detection of minimal residual disease.

Multiple technologies and platforms have been developed to extract, amplify and analyze these circulating biomarkers. Next-generation sequencing (NGS), with its high throughput and sensitivity, stands out as a powerful tool for profiling ctDNA, uncovering actionable mutations and guiding targeted therapies (Mouliere *et al.*, 2018). Digital polymerase chain reaction (dPCR) offers precise quantification of mutant alleles, enhancing the detection sensitivity of minute amounts of ctDNA (Schwarzenbach, Hoon, and Pantel, 2011). These technologies collectively form the arsenal of liquid biopsy, enabling the elucidation of intricate molecular signatures in a minimally invasive manner.

The clinical applications of liquid biopsy are vast and impactful. Early detection of cancer, a critical factor in improving patient outcomes, is facilitated by the ability to detect minute amounts of ctDNA even before clinical symptoms manifest (Cohen *et al.*, 2018). Liquid biopsy also plays a pivotal role in selecting targeted therapies based on the specific genetic alterations identified, thereby personalizing treatment strategies and improving therapeutic efficacy (Tong *et al.*, 2019). Furthermore, the monitoring of treatment response and the detection of minimal residual disease contribute to a comprehensive approach to cancer management.

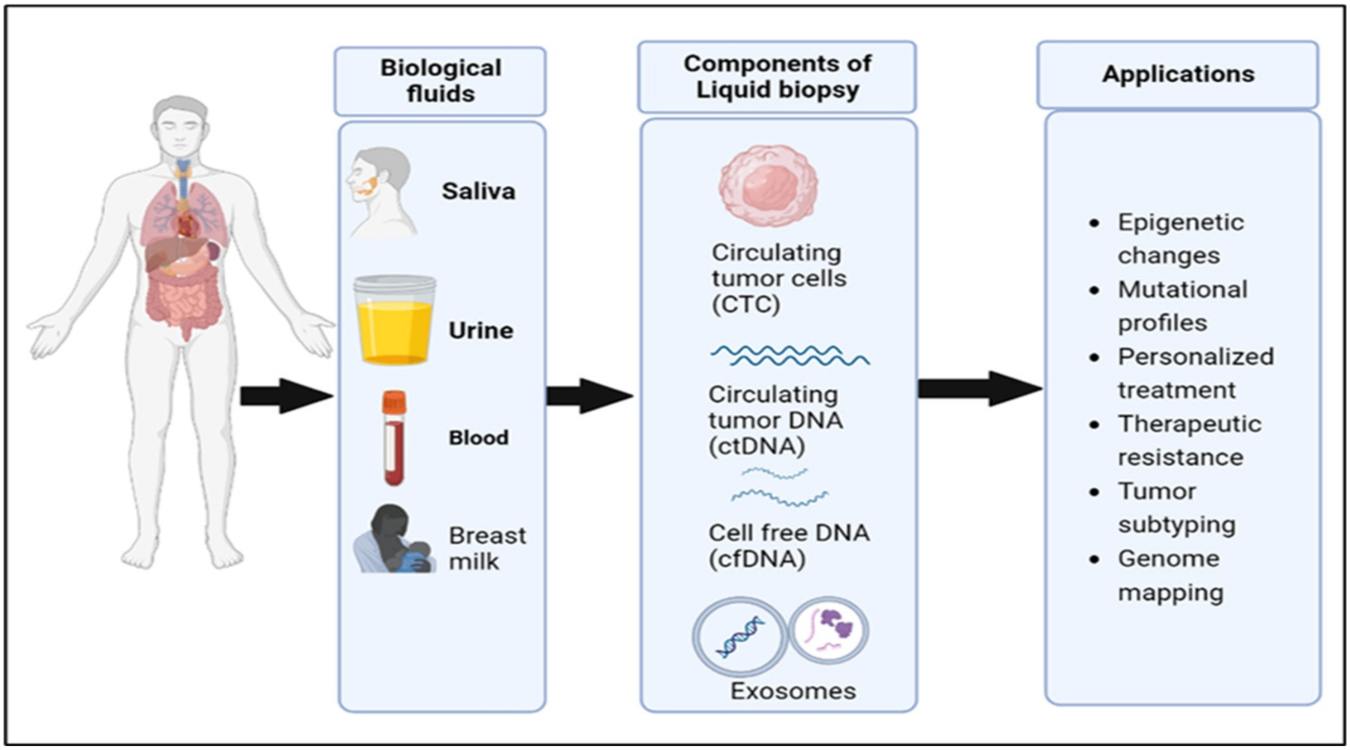

**Fig. 2**: Sampling fluids, components and applications of liquid biopsy.

As with any transformative technology, liquid biopsy is not without challenges. Sensitivity and specificity issues, standardization concerns and the need for large-scale validation studies are among the hurdles that must be addressed for widespread clinical implementation. However, ongoing research and technological advancements continue to refine and enhance the utility of liquid biopsy in the realm of cancer diagnostics.

This review aims to provide a comprehensive understanding of the principles, technologies, clinical applications and challenges associated with liquid biopsy. By critically examining the current state of liquid biopsy and its comparative advantages over traditional methods, we illuminate the path towards a brighter future in cancer diagnosis and management.

## Principles of Liquid Biopsy

Liquid biopsy, a revolutionary approach in cancer diagnostics, is grounded in the analysis of circulating biomarkers released by tumours into the bloodstream. This non-invasive method provides a dynamic and real-time representation of the genomic landscape of cancer, offering a comprehensive view of molecular alterations. The primary components of liquid biopsy include circulating tumour DNA (ctDNA), circulating tumour cells (CTCs) and extracellular vesicles like exosomes.

CtDNA, perhaps the most well-studied component, consists of fragmented DNA shed by tumour cells into circulation. These fragments carry genetic mutations and alterations reflective of the primary tumour's genomic profile (Siravegna *et al.*, 2017). The analysis of ctDNA provides valuable insights into the genetic heterogeneity of tumours, aiding in early detection and monitoring treatment response.

CTCs are intact cancer cells that have detached from the primary tumour and entered the bloodstream. Their identification and analysis provide information on tumour biology and can be indicative of metastatic potential (Tong *et al.*, 2019). Additionally, the analysis of extracellular vesicles, particularly exosomes, allows for the examination of microRNA and other molecular cargo, expanding the repertoire of biomarkers accessible through liquid biopsy (Mouliere *et al.*, 2018).

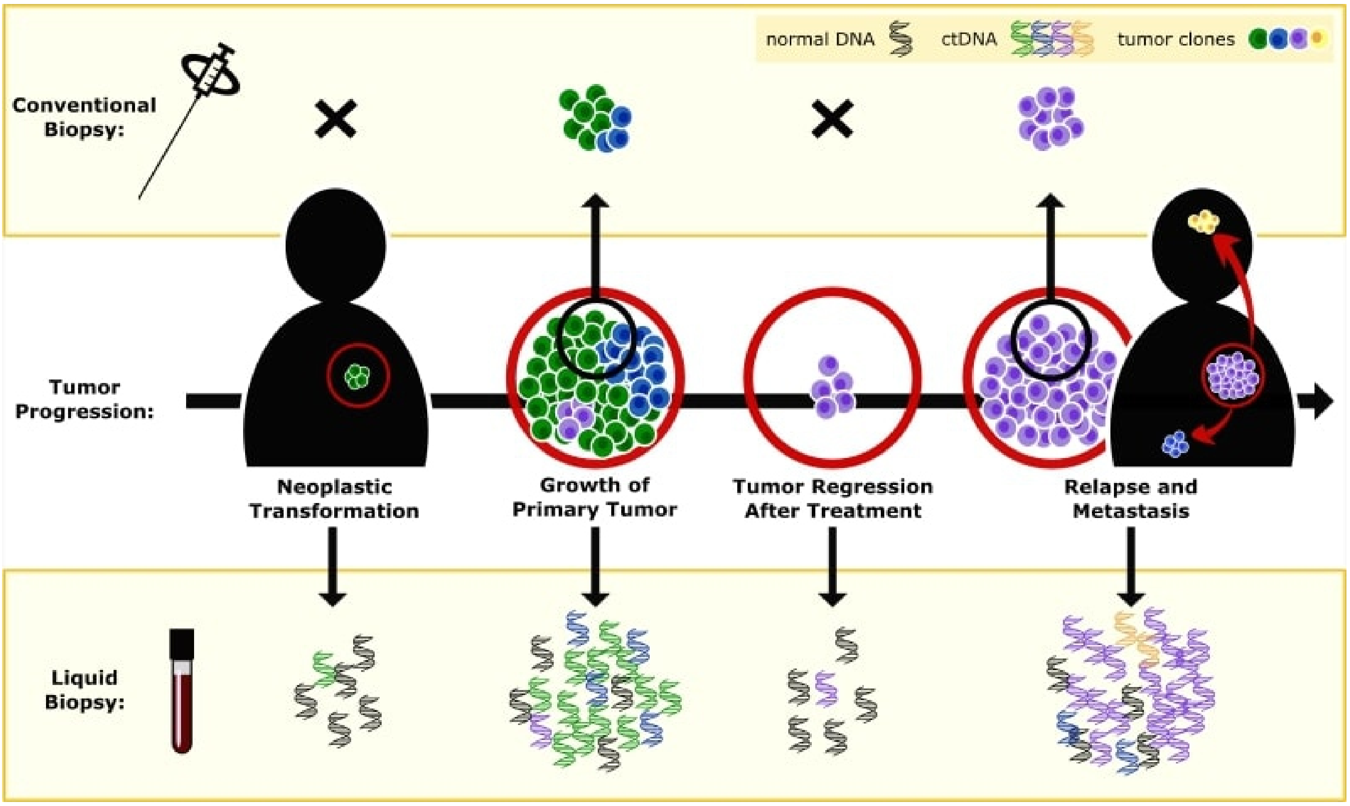

**Fig. 3:** Applications of ctDNA for liquid biopsy.

These circulating biomarkers are isolated from a simple blood draw, making liquid biopsy an attractive and minimally invasive alternative to traditional tissue biopsies. The analysis of ctDNA, CTCs and exosomes is facilitated by a range of technologies, with next-generation sequencing (NGS) standing out for its high throughput and ability to detect low-frequency mutations (Mouliere *et al.*, 2018). Digital polymerase chain reaction (dPCR) complements NGS by offering precise quantification of mutant alleles, enhancing sensitivity (Schwarzenbach, Hoon, and Pantel, 2011).

To simplify, the principles of liquid biopsy involve the identification and analysis of circulating tumour components in the bloodstream, providing a real-time, minimally invasive and comprehensive approach to understanding the genomic landscape of cancer. These principles underpin the transformative potential of liquid biopsy in reshaping cancer diagnostics and management.

## Technologies and Platforms

The implementation of liquid biopsy relies on a diverse array of technologies and platforms that enable the extraction, amplification and analysis of circulating biomarkers. These methodologies play a pivotal role in unlocking the potential of liquid biopsy for cancer diagnostics.

Next-generation sequencing (NGS) stands out as a cornerstone technology, offering high-throughput sequencing of DNA. NGS enables the comprehensive analysis of circulating tumour DNA (ctDNA) by detecting genetic alterations, including point mutations, insertions, deletions and copy number variations (Mouliere *et al.*, 2018). The sensitivity of NGS allows for the identification of low-frequency mutations, crucial for understanding tumour heterogeneity and guiding targeted therapies.

Digital polymerase chain reaction (dPCR) complements NGS by providing precise quantification of mutant alleles in a sample. This technology enhances the sensitivity of liquid biopsy, allowing for the detection of minimal amounts of ctDNA (Schwarzenbach, Hoon and Pantel, 2011). The ability to quantify mutated alleles accurately is particularly valuable in monitoring treatment response and detecting residual disease.

Mass spectrometry (MS) has also emerged as a powerful tool in liquid biopsy. It enables the identification and quantification of proteins and peptides in circulating biomarkers, contributing to a more comprehensive analysis of the tumour microenvironment (Mouliere *et al.*, 2018). MS can be employed to study proteins associated with exosomes, shedding light on the role of extracellular vesicles in cancer progression.

Microfluidic devices represent another innovative platform for liquid biopsy. These devices allow for the isolation and characterization of circulating tumour cells (CTCs) based on their physical and biological properties (Tong *et al.*, 2019). Microfluidic technologies enhance the efficiency of CTC capture, facilitating downstream analysis for molecular characterization.

While each technology has its strengths and limitations, their integration offers a multi-modal approach to liquid biopsy, providing a more comprehensive understanding of the molecular landscape of cancer. The continuous refinement and combination of these technologies contribute to the ongoing evolution of liquid biopsy as a transformative tool in cancer diagnostics.

## Clinical Applications

The clinical applications of liquid biopsy are broad-ranging, offering a transformative impact on cancer diagnosis and management. This review explores the diverse applications of liquid biopsy in clinical settings, emphasizing its significance in early detection, treatment selection, monitoring treatment response and detecting minimal residual disease.

Early detection of cancer is a critical factor in improving patient outcomes and liquid biopsy excels in this domain. The ability to detect circulating tumour DNA (ctDNA) in blood provides a unique opportunity for early diagnosis, often preceding clinical symptoms (Cohen *et al.*, 2018). Liquid biopsy allows for the identification of minimal amounts of ctDNA shed by tumours, enabling the detection of cancer at earlier, more treatable stages.

In the realm of personalized medicine, liquid biopsy plays a pivotal role in guiding treatment decisions. By analyzing the genetic alterations present in ctDNA, clinicians can identify actionable mutations and select targeted therapies that are most likely to be effective (Tong *et al.*, 2019). This approach represents a paradigm shift towards precision medicine, tailoring treatments to the individual genomic profile of each patient.

Monitoring treatment response is another crucial application of liquid biopsy. The dynamic nature of ctDNA enables real-time tracking of genetic changes in response to treatment. This allows clinicians to assess the effectiveness of therapies and make informed decisions about treatment adjustments (Siravegna *et al.*, 2017). Liquid biopsy thus contributes to a more personalized and adaptive approach to cancer care.

Furthermore, liquid biopsy facilitates the detection of minimal residual disease, providing insights into the presence of residual cancer cells after treatment. The identification of minimal residual disease is essential for predicting the risk of disease recurrence and guiding post-treatment surveillance strategies (Siravegna *et al.*, 2017).

Despite these promising applications, challenges such as sensitivity, specificity and standardization need to be addressed for widespread clinical adoption. Nevertheless, the multifaceted clinical applications of liquid biopsy underscore its potential to revolutionize cancer diagnostics and usher in a new era of precision medicine.

## Challenges and Future Directions

While liquid biopsy holds immense promise, several challenges must be addressed to unlock its full potential in cancer diagnostics. Sensitivity and specificity remain critical concerns, as the detection of circulating tumour DNA (ctDNA) and other biomarkers often involves discerning low-abundance signals amidst the background of normal cell-free DNA (Mouliere *et al.*, 2018). Improving the analytical sensitivity of liquid biopsy assays is crucial to ensure reliable detection, especially in early-stage cancers.

Standardization is another challenge that needs careful consideration. The lack of standardized protocols for sample collection, processing and analysis introduces variability across studies and limits the comparability of results (Mouliere *et al.*, 2018). Establishing consensus guidelines and benchmarks for liquid biopsy procedures will enhance reproducibility and reliability, fostering the integration of this technology into routine clinical practice.

The identification of specific and robust biomarkers for different cancer types is an ongoing challenge. While ctDNA provides a wealth of information, identifying tumour-specific alterations amidst the vast genomic landscape is complex. Future research should focus on the discovery and validation of novel biomarkers that improve the specificity of liquid biopsy for different cancer types (Siravegna *et al.*, 2017).

Looking ahead, the integration of artificial intelligence (AI) and machine learning (ML) in data analysis holds great promise for overcoming current challenges. AI can enhance the interpretation of complex genomic data, improving the accuracy and efficiency of liquid biopsy analyses (Siravegna *et al.*, 2017). Implementation of these technologies will require collaborative efforts between computational biologists, clinicians and industry stakeholders.

In terms of future directions, exploring the potential of liquid biopsy in monitoring treatment resistance and guiding the selection of subsequent therapies is a promising avenue. Additionally, investigating the utility of liquid biopsy in other diseases beyond cancer, such as infectious diseases and autoimmune disorders, broadens its scope and impact (Siravegna *et al.*, 2017).

While challenges exist, the future of liquid biopsy is promising. Addressing sensitivity, specificity and standardization concerns, coupled with advancements in AI-driven data analysis, will pave the way for widespread adoption, ultimately reshaping the landscape of cancer diagnostics.

## Comparative Analysis with Traditional Methods

A critical aspect of evaluating the transformative potential of liquid biopsy in cancer diagnostics is conducting a comparative analysis with traditional methods, particularly tissue biopsy. Traditional biopsy, while considered the gold standard, is not without limitations. Liquid biopsy emerges as a promising alternative, addressing some of these challenges.

Firstly, the invasiveness of tissue biopsy raises concerns related to patient discomfort, potential complications and the limited ability for serial sampling. In contrast, liquid biopsy offers a non-invasive approach, extracting circulating tumour DNA (ctDNA) and other biomarkers from a simple blood draw, providing a dynamic and less intrusive means of monitoring the evolving tumour landscape (Siravegna *et al*., 2017).

Tissue biopsy is inherently spatially limited and may not capture the full genetic heterogeneity of a tumour, leading to potential sampling bias. Liquid biopsy, by virtue of analyzing ctDNA shed by tumours into the bloodstream, offers a more comprehensive representation of the tumour's genomic profile, overcoming the spatial constraints of tissue biopsy and providing a holistic view of tumour heterogeneity (Siravegna *et al*., 2017).

Moreover, the temporal dynamics of cancer, especially during treatment, are challenging to capture with traditional biopsy methods. Liquid biopsy, with its ability to perform real-time monitoring of ctDNA, enables clinicians to track treatment response, detect minimal residual disease and identify emerging resistance mechanisms, providing a more dynamic and adaptive approach to cancer management (Mouliere *et al*., 2018).

While liquid biopsy presents a paradigm shift, challenges such as sensitivity, specificity and standardization need to be addressed for seamless integration into clinical practice. The comparative advantages of liquid biopsy in terms of patient comfort, real-time monitoring and comprehensive profiling position it as a transformative complement to traditional methods, offering new dimensions in the diagnosis and management of cancer.

## Ethical and Regulatory Considerations

The integration of liquid biopsy into routine clinical practice necessitates a careful examination of ethical and regulatory considerations to ensure responsible and equitable implementation.

Patient consent is a cornerstone of ethical considerations in liquid biopsy research and clinical use. Given the evolving nature of this field, it is imperative to obtain informed consent that clearly outlines the purpose of the liquid biopsy, the potential implications of the results and how the data will be used. Ensuring that patients are well-informed about the benefits and risks is essential for upholding the principles of autonomy and respect for individuals (Schwarzenbach, Hoon, and Pantel, 2011).

Data privacy and confidentiality are paramount ethical concerns in the era of liquid biopsy. The highly sensitive nature of genomic information demands robust safeguards to protect patient privacy. Researchers and clinicians must adhere to stringent data security measures and comply with relevant data protection regulations to prevent unauthorized access and use of patient information (Schwarzenbach *et al*., 2011).

Regulatory frameworks play a pivotal role in shaping the responsible use of liquid biopsy technologies. Health regulatory bodies need to establish clear guidelines for the validation, standardization and quality assurance of liquid biopsy assays. These regulations should address issues related to analytical validity, clinical validity and clinical utility to ensure the reliability and utility of liquid biopsy results in guiding patient care (Siravegna *et al.*, 2017).

Moreover, as liquid biopsy technologies evolve, ongoing ethical discourse is essential. Continuous engagement with stakeholders, including patients, clinicians, researchers and policymakers, fosters an ethical and inclusive approach to the development and implementation of liquid biopsy in cancer diagnostics. This approach encourages transparency, accountability and responsiveness to the diverse needs and perspectives of the individuals involved.

The ethical and regulatory considerations are integral to the responsible advancement of liquid biopsy technologies. By prioritizing patient consent, data privacy and adherence to regulatory standards, the integration of liquid biopsy into clinical practice can be ethically sound, equitable and aligned with the principles of patient-centered care.

## Conclusion

The liquid biopsy emerges as a transformative force in cancer diagnosis, offering a paradigm shift from traditional tissue biopsies. Through the analysis of circulating tumour DNA (ctDNA), circulating tumour cells (CTCs) and other biomarkers, liquid biopsy provides a non-invasive, dynamic and comprehensive approach to understanding the genomic landscape of cancer. The metaphorical ‘new sunshine’ encapsulates the hope and brightness liquid biopsy brings to the field, revolutionizing early detection, treatment selection and monitoring of cancer.

While challenges like sensitivity and standardization persist, ongoing advancements in technologies and regulatory frameworks are paving the way for widespread adoption. Ethical considerations, including patient consent and data privacy, are integral to ensuring responsible implementation. As we navigate the intricate landscape of liquid biopsy, its comparative advantages over traditional methods underscore its potential to reshape the future of cancer diagnostics, offering personalized and adaptive strategies for improved patient outcomes.

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**RESEARCH ARTICLE**

# Impact of nanoparticles on various aspects of plant functional biology

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## Abstract

More than 60% of the global population depends on agriculture for their living, making it the backbone of the majority of developing nations where it provides food for people both directly and indirectly. Agricultural growth depends heavily on nanotechnology, which has received substantial governmental financing, but progress has been slow. Excellence in nanoparticle technology has been motivated by recent developments and discoveries in chemistry and nanoscience, with colossal implicative insinuations in agriculture. To reduce agricultural waste and greenhouse gas emissions, there has been a rapid trend toward green nanotechnology. The size and surface characteristics of the nanoparticles play a significant role in how well they are absorbed by plant surfaces. The use of nano-zeolites improves the soil environment for crop enlargement, lowers the cost of importing farm technology in the future and helps to maintain a sustainable agricultural industry. Nanotechnology has a vast array of possible uses and benefits. Through improved monitoring and focused action, increasing crop productivity through precision agriculture supported by nanotechnologies is desirable for optimising output and minimising inputs. Crops can more effectively use herbicides, fertilisers and water due to nanotechnology. Because certain nanoparticles improved plant tolerance to a variety of biotic and abiotic challenges, it is anticipated that their use in agriculture will continue to increase. Studies on the impact of these nanoparticles on genetic material, which is crucial for improving crops genetically, are quite rare. Increasing our understanding of genetic alterations is extremely important and must be addressed immediately. Additionally, various interactions between several plant species and the nanoparticles were seen. Field crops must be modified to satisfy the future pabulum demand and all modifications brought about by nanoparticles in plant morphological features, physiological parameters and gene expression are crucial.

## Introduction

A creative, cutting-edge and scientific technique called nanotechnology enables the design, manipulation and creation of useful nanomaterials. The agriculture industry is the foundation of emerging nations. Therefore, there is an urgent need to create a technology that will improve current agriculture by making it more fruitful, economical and environmentally friendly. The use of nanotechnology in agriculture is growing

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quickly to improve food quality, reduce the need for agricultural inputs, modify nutritional concentrations and extend shelf life (Singh *et al*., 2015; Sunghee 2017; Ali *et al*., 2018; Mali *et al*., 2020). The use of nanotechnology in plant functional biology is growing as a result of the injunctive demand for higher crop productivity by a continuously expanding population. The term "nanotechnology" denotes the molecular and sub-molecular technology on the nanoscale, classically more minute than 100nm (Abraham *et al*., 2008; Prasad *et al*., 2014). In combination, nanotechnology may offer précised agricultural techniques for increasing crop productivity with the use of sensors and monitoring devices, so fostering ecumenical food production and demonstrating good influence on the agricultural industry (Mukhopadhyay 2014). The avoiding circumstances have a great impact on plant growth and germination. Growth and germination may be encouraged or discouraged by the presence of a chemical (Shojaei *et al*., 2009).

By developing emerging techniques like precision farming, improving plants' ability to absorb nutrients, more efficient and targeted input usage, disease detection and management and more, nanotechnology will revolutionise the agricultural and food industries. By using nanoclays and zeolites, fertiliser application efficiency may be increased while soil fertility is created by letting go of specially formulated nutrients (Joshi *et al*., 2018). Furthermore, it has been shown that using nanoparticles advances plant tolerance to a variety of biotic stressors, including salt, heat and drought. Since there are various ways that nanoparticles might improve crop development and production, the unfavourable impacts of specific types of nanoparticles have been identified and optically explored. Therefore, being aware of the benefits and drawbacks is crucial for the effective use of nanoparticles (El-Moneim *et al*., 2021). Given the enormous potential of nanoparticles (NPs) to affect almost all sectors and traditional processes, nanotechnology has seen extraordinary progress. NPs have been widely used in agronomy to improve the physiology, development and nutritional value of goods derived from plants. The positive physiological changes following NPs treatments include increased photosynthetic rates, chlorophyll and proline content, improved homeostasis, hormone balance and nutritional absorption. On the other hand, NPs do not always have an influence on plants that is beneficial or negative and no physiological influences are sometimes visibly canvassed. This review presents the progressions made in the field of nanotechnology regarding the impact of various NPs on plant physiology/functional biology. Different branches of plant sciences can benefit from an understanding of the intricate dynamics of nanoparticle in plants. That can help determine agricultural sustainability in exchange (Ahmad *et al*., 2022).

## Nanoparticles improving plant functional biology/physiology

The use of nanoparticles controls germination, acclimation, and plant growth (Banerjee *et al*., 2021). Additionally, nanoparticles may have increased the rate of photosynthetic activity, promoted the decomposition of organic materials and enhanced the absorption of mineral elements (Khot *et al*., 2012). The right quantities of nanoparticles may penetrate into a plant's system and impact its metabolic processes, which has beneficial effects on plant development (Parisi *et al*. 2015; Zaytsena and Neumann 2016). Additionally, nanotechnology offers outstanding answers for a growing variety of environmental problems. For instance, the invention of nanosensors offers vast potential for improving plant defences against disease and visual discernment of environmental stress

(Afsharinejad *et al.*, 2016; Kwak *et al.*, 2017). Therefore, such ongoing advancements in nanotechnology, with a focus on problem-solving and the creation of cooperative strategies for sustainable agricultural growth, have a tremendous potential to bring about wide-ranging, egalitarian advantages. Nano-fertilizers may be able to increase crop engendering in currently unfavourable settings, according to advances in nanomaterial engineering. By using the nanoparticles as fertiliser topically, it has been observed that engenderment has been altered (Raliya and Tarafdar, 2013). Numerous nanomaterials are created for absorption, translocation, storage and generally for their effects on growth. These materials are often based on metal or carbon (Nair *et al.*, 2010). It has been shown that some crops, such as soybean (Agrawal and Rathore, 2014), spinach (Gao *et al.*, 2006), and peanut, benefit from metal-predicated nanomaterials that improve a variety of physiological characteristics (Giraldo *et al.*, 2014). Silver nanoparticles (SN) (0-80 mg $L^{\ 1}$) were shown to postpone the senescence process in the petals of two separate cultivars of *Pelargonium zonale* (Foxi and Flowerfairy) under dark condition, according to Hatami and Ghorbanpour's 2014 research. The activities of (APX), PerOxiDase (POD), Superoxide Dismutase (SOD), FELINE, and b-GLUcosidase (b-GLU) were measured by these researchers, as well as the concentration of leaf protein, MDA and ethylene, petal abscission and the Membrane Stability Index (MSI).

## Nanoparticles regulating plant growth and germination

Gram (*Cicer arietinum*) plant is grown in an aqueous solution that contains various concentrations of water-soluble carbon-nano-onions from germination to seed production (WsCNO). The effect of WsCNO on the growth of gram plants can be persistent in terms of shoot length, branching, number of roots and length (Sonker *et al.*, 2012). After the incorporation of CNP, Saxena *et al.*, (2014) found favourable benefits on agricultural yield of wheat plants demonstrating the increased shoot lengths and growth rate. Use of multi-walled carbon nanotubes (MWCNT) improved the biomass engenderment of tomato plants and stimulated the germination of seeds in barley, wheat, maize and garlic (Khodakovskaya *et al.*, 2009; Joshi *et al.*, 2018; Shang *et al.*, 2019). According to research by Singh *et al.*, (2015), $SiO_2$ and $TiO_2$ nanoparticles are useful for seed germination in a variety of agricultural plants. Recent research has shown that nanoparticles can puncture the seed coat, enabling the absorption of nutrients and water (Banerjee *et al.*, 2016). *Lycopersicum esculentum* seed germination was altered by using 8 g/L of 12 nm nano-silicon oxide (Siddiqui *et al.*, 2014). Wheat seedlings were grown in quartz sand with 500-8000 mg/kg of citrated-coated magnetite NPs (CCM-NPs) (Lopez-Luna *et al.*, 2016). The outcomes revealed that germination was resistant to the NPs treatments. *A. thaliana* was not responsive to $Fe_2O_3$ NPs (Marusenko *et al.*, 2013; Mukherjee *et al.*, 2014) Tomato seeds (*Lycopersicon lycopersicum*) were subjected to CoFe2O4 nanoparticles in hydroponics at concentrations ranging from 0 to 1000 mg/L. When compared to control (99%), there was no significant difference in germination across any of the treatments (Lopez- Moreno *et al.*, 2016). The germination of the seeds of the medicinal tree species *Boswellia ovalifoliolata* was favourably impacted by Ag-NPs (Savithramma *et al.*, 2012). Water hyssop (*Bacopa monnieri*) cultivated hydroponically was tested for sensitivity to biologically produced Ag-NPs, and seed germination was considerably impacted (Krishnaraj *et al.*, 2012). According to Parveen and Rao (2014), Ag-NPs improved pearl millet germination (*Pennisetum glaucum*). Another investigation on old rice seeds (*Oryza sativa* L.) reinforced that Ag-NPs at 5 and 10 mg/L modified the seedling vigour and germination performance when primed with phyto-synthesized Ag-NPs (Hussain *et al.*, 2017; Mahakham *et al.*, 2017) investigated the impact of three metallic nanoparticles (Ag, Cu, and Au) on wormwood seed germination as well as other factors (*Artemisia absinthium*). Compared to the other two metal-NPs, Ag-NPs were the most effective at enhancing seed germination (i.e., 99%) 35 days after inoculation. In certain cases, species including *Brassica juncea, Boswellia ovalifoliolata, Gloriosa superba*, and *Arabidopsis thaliana* exhibit improved seed germination when treated with Au-NPs (Barrena *et al.*, 2009; Arora *et al.*, 2012; Savithramma *et al.*, 2012; Kumar *et al.*, 2013; Gopinath *et al.*, 2014). According to Shah and Belozerova (2009), the germination of lettuce (*Lactuca sativa*) seeds was favourably impacted by low quantities of Au-NPs and a cumulation of Cu-NPs and Au-NPs. Similar to this, Au-NPs created by green synthesis from the fruit extract of *Terminalia arjuna* improved the germination of flame lily (*G. superba*), a critically endangered medicinal plant (Gopinath *et al.*, 2014). Si-NPs administration improved tomato seed germination and under NaCl stress, the antioxidant system was also boosted (Haghighi *et al.*, 2012; Siddiqui and Al-Whaibi, 2014). Similar to this, $SiO_2$-NPs were tagged by Suriyaprabha *et al.*, (2012) for improved conductivity to the growth medium and nutrient accessibility to maize seedlings. Additionally, it has been shown that $SiO_2$-NPs (sodium silicate, $Na_2SiO_3$, and $SiO_2$) have beneficial effects on the germination of broad bean, rice, thyme and lettuce (Shah and Belozerova, 2009; Adhikari *et al.*, 2013; Wu *et al.*, 2014; Roohizadeh *et al.*, 2015; Abbasi Khalaki *et al.*, 2016) reported a positive effect of $TiO_2$-NP on seed germination when bombarding lettuce seeds using the electrospray technique. These positive effects of $TiO_2$-NPs were discovered to operate in a time-dependent manner in *Prunus mahaleb* and wheat plant cv. Parsi (Mahmoodzadeh and Aghili, 2018). In addition, Mahmoodzadeh *et al.*, (2013) found favourable benefits of $TiO_2$-NPs for boosting radicle and plumule development of canola seedlings (Yang *et al.*, 2006). Cucumber, radish, maize, lettuce, rapeseed and ryegrass seed germination and root development are positively influenced by ZnO-NPs (Milewska-Hendel *et al.*, 2016). Germination is dependent on the plant type and Fe form, according to an optical survey (Libralato *et al.*, 2016).

## Nanoparticles response in transpiration

Additionally, it has been claimed that nanostructured silicon dioxide reduces plant transpiration rates, changes the colour of the plant's leaves, and encourages the growth of new shoots (Avestan *et al.*, 2016). Yeo *et al.* observed that treatment with silicon caused *Oryza sativa* to have a reduced salt absorption and transpirational bypass flow (Yeo *et al.*, 1999). In compared to the control, the wheat plant's shoot portion grows more quickly when water-soluble CNP is present. The study's CNP samples ranged in concentration from 10 to 150 mg/L, however 50 mg/L was determined to provide the best growth rates since higher concentrations of CNP may be detrimental to plant development. On the other hand, Chakravarty *et al.* (2015) reported on the use of graphene quantum dot (GQD) for growth improvement in the coriander (*Coriandrum sativum* L.) and garlic plant (*Allium sativum*). Additionally, graphene exposure had a highly phytotoxic effect on plants, stunting the development and biomass of cabbage, tomatoes and red spinach while also increasing ROS, cell death and necrotic lesions. Similar to previous CBNMs, the amount of plant damage was related to the dose, the duration of exposure and the kind of plant involved (Begum *et al.*, 2011). It is examined how GQD affects the physiological reproduction of coriander and garlic plants, including the development of their leaves, roots, shoots, flowers and fruits containing seeds. The size of the leaves and the quantity of flowers are visually shown to be increased in coriander plants that have received GQD treatment. Additionally, it was visually observed that GQD increased the average length of coriander roots in comparison to the plant treated without GQD.

## Nanoparticles in organ formation

Silver nanoparticle applications promote initiation start of roots in cabbage and maize (Pokhrel *et al.*, 2013). ZnO nanoparticle application affected root-shoot development, seed germination, and increased biomass engendering in peanut and chickpea plants, respectively. Wheat seed germination is promoted by ZnO nanoparticles (Prasad *et al.*, 2012; Ramesh *et al.*, 2015).

## Nanoparticles in plant mineral nutrition

According to Manjaiah *et al.* (2019), nano zeolite can improve nutrient availability over the long term and promote plant germination and development. Wheat has been treated with natural polymer, such as chitosan NPs, to regulate the release of nitrogen, phosphorus, and potassium by foliar absorption (Abdel-Aziz *et al.*, 2016). In terms of environmental contamination, using organic NPs is more acceptable. However, stronger proof is required to demonstrate their efficacy benefits for nutrient supply over conventional fertilisation techniques (Liu and Lal, 2015; Sanzari *et al.*, 2019).

## Nanoparticles in regulating photosynthesis and pigment concentrations

According to a study, adding $TiO_2$ nanoparticles improved spinach's photosynthetic performance by raising the amount of protein and chlorophyll in the leaf. $TiO_2$ nanoparticle treatment in spinach further promotes the activity of the Rubisco enzyme (Zheng *et al.*, 2005). Similarly, Au nanoparticles have an impact on plant height and leaf count in relation to increased agricultural yield (Gopinath *et al.*, 2014). Pearl millet's protein and chlorophyll concentrations increased when green Zn nanoparticles were applied to the plant and shoot development and productivity were also improved (Sonker *et al.*, 2012). Nano $FeSo_4$ inhibited——net carbon dioxide ($CO_2$) assimilation rate, sub-stomatal $CO_2$ concentration (Ci), chlorophyll content, maximum photochemical efficiency of photosystem II (Fv/Fm) and iron (Fe) content. Si nano-particles (SiNPs) have recently been investigated for their potential to effectively reduce UV-B induced stress in wheat (Tripathi *et al.*, 2017). According to reports, $TiO_2$ nanoparticles (less than 4% concentration) improve spinach's ability to fix nitrogen and stimulate photosynthesis, improving the plant's overall growth efficiency (Zheng *et al.*, 2005). In doses greater than 500 mg kg$^{1}$ nZVI, chlorophyll abbreviation was substantially different from control nevertheless, the strongest chlorophyll inhibition was visually assessed at 1000 mg/kg$^{1}$ nZVI (Wang *et al.*, 2016). By activating Rubisco, nano-anatase $TiO_2$ improves the photosynthetic uptake of carbon, which might stimulate Rubisco carboxylation and increase plant development (Gao *et al.*, 2006). After exposure to $TiO_2$ NPs, Ze *et al.*, (2011) discovered a corresponding rise in the expression of the light-harvesting intricate II (LHCII) b gene in *Arabidopsis*.

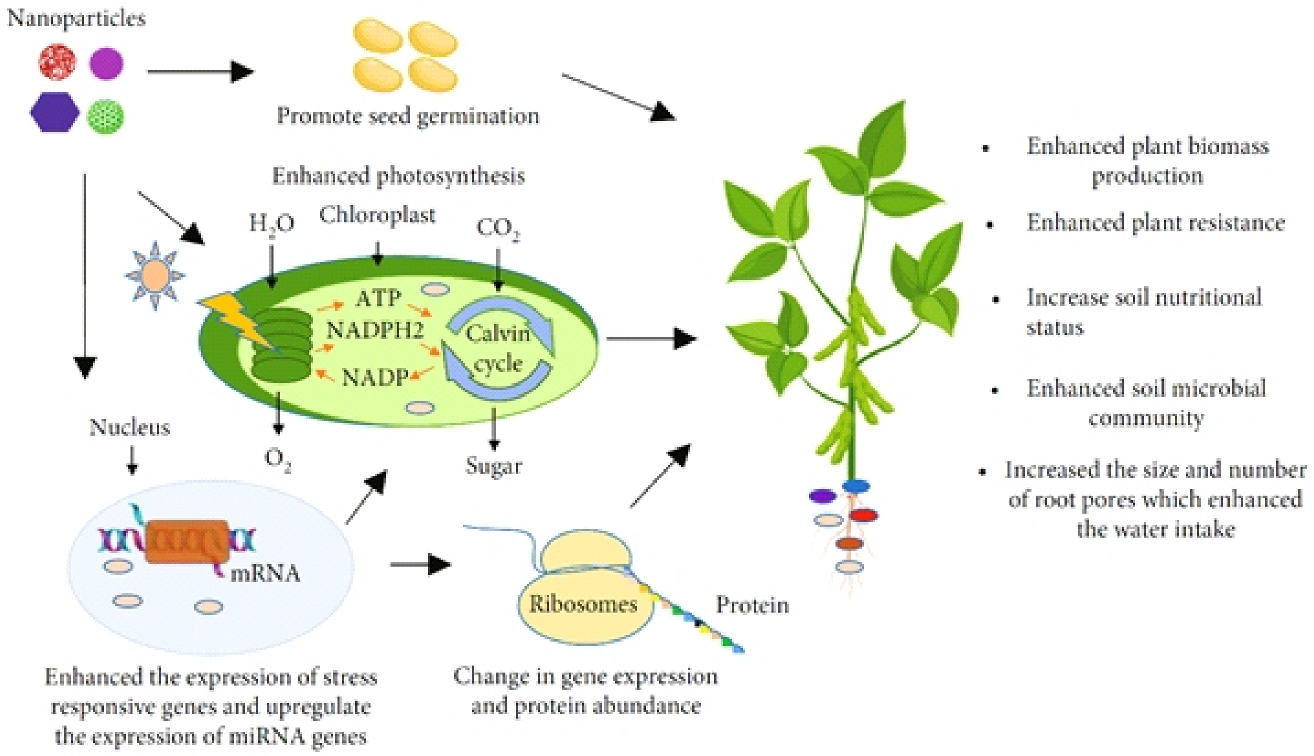

**Fig 1: Positive effects of nanoparticles on plant growth and development. The optimum concentration of nanoparticles causes an alteration in different physiological processes to increase seed germination and photosynthesis of the plants. Further, the nanoparticles alter the gene expression of different genes and miRNAs that have a positive impact on stress tolerance and plant biomass. (adapted from Ali *et al.*, 2021)**

The thylakoid membrane's high LHCII level increased the chloroplast's capacity to absorb light. The effect of nano-anatase on the molecular mechanism of carbon reaction was investigated by Linglan *et al.* in 2008. They proposed that the nano-anatase stimulates RCA, a marker gene for Rubisco activase, and boosts Rubisco activase protein levels and activities, which improve Rubisco carboxylation and the rapid photosynthetic carbon reaction. The gene profiles of Arabidopsis were examined by Wang *et al.* (2016) in replication to ZnO NPs and ZnSO4. In soybean plants, carotenoids content decreased at 400 mg/L CuO NPs but chlorophyll started declining at 400 mg/L. (Nair and Chung, 2014a). CuO NPs, on the other hand, shortened carotenoids and chlorophylls in mustard, whereas Cu predicated NPs had no effect on chlorophyll genesis in cilantro (Nair and Chung, 2015). (Zuverza-Mena *et al.*, 2015). According to Nair *et al.* (2014), in soybean plants, carotenoids concentration decreased at 400 mg/L CuO NPs, while chlorophyll started declining at 400 mg/L, however in mung bean plants, carotenoids stayed unaltered and chlorophyll reduction started at 100 mg/L CuO NPs (Nair and Chung, 2014a). (Zuverza-Mena *et al.*, 2015). In addition to applying NPs, there are several additional possible strategies to react to stressors in plants. This may help to reduce, photoreduction activity, the oxygen evolution rate of chloroplasts and the instability of the thylakoid membrane (Du *et al.*, 2011; Khan *et al.*, 2017). An amendment in Hill reaction, oxygen evolution, Rubisco expression, photosynthetic rates, chlorophyll content, carotenoids content and proline contents, along with leaf relative water content, have been documented for *C. arietinum, S. lycopersicum, Moringa peregrina, C. pepo, B.*

*chinensis Ocimum basilicum, Helianthus annuus, , G. max* and *Pelargonium zonale* upon application of $SiO_2$, ZnO and $Fe_3O_4$, ZnO, $TiO_2$, $Na_2SeO_4$, hydroxyapatite, $TiO_2$, $Al_2O_3$, Au, and Ag-NPs (Du *et al*., 2011; Khan *et al*., 2017; Venzhik *et al*., 2021). Nevertheless, plants like kidney bean, corn, rice, pepper, cucumber, zucchini, and lettuce were unaffected when Au and $CeO_2$ Instead, the addition of $TiO_2$, Au, CuO, ZnO, $CeO_2$, and NPs had a detrimental impact on the photosynthetic parameters of *Clarkia unguiculata, H. vulgare, O. sativa, Gossypium herbaceum, C. sativus, T. aestivum, R. sativus,* and *Ulmus elongata* seedlings (Du *et al*., 2011; Venzhik *et al*., 2021). However, the photosynthetic capability of plants including kidney bean, corn, rice, pepper, cucumber, zucchini and lettuce was unaffected by the application of Au and $CeO_2$ NPs (Venzhik *et al*., 2021). Given that photosynthesis is an involute process, the variety and *V. unguiculata* have led to improvements in growth, seed weight, yield, and quality metrics (Ditta and Arshad, 2016).

## Nanoparticles in phytohormonal regulation

Indole-3-acetic acid (IAA) and abscisic acid (ABA) concentrations were changed as a result of exposing cotton and Bt-cotton to CuO NPs (Le Van *et al*., 2016). As a result, it was discovered that using Ag-NPs decreased the expression of genes involved in the replication of gibberellic acid, ethylene, cytokinins, auxins, and abscisic acid (ABA) in rice callus (Landa, 2021). Ag-NPs also increased the levels of indole-3-acetic acid, gibberellin GA3 and total cytokinins while decreasing the level of abscisic acid (Landa, 2021). It's interesting to note that different research reported using phytohormones to reduce the toxicity of NPs, and that the use of indole-3-acetic acid (IAA) on *Pisum sativum* reduced the toxicity of CuO-NPs (Ochoa *et al*., 2017). This could be an effective method for reducing stress, but if using NPs to promote better crop development results in hazardous side effects that must be countered by the use of phytohormones, it would defeat the purpose of using NPs in the first place. Applying CuO-NPs to cotton and Bt-cotton crops significantly changed the concentrations of IAA and ABA (Zuverza-Mena *et al*., 2017).

## Nanoparticles in mitigating abiotic stresses in plants

Overabundances of metal-predicated NPs in cultivated interfere with the electron transport chain and damage the ROS detoxification system, (Dimkpa *et al*., 2013; Faisal *et al*., 2013; Pakrashi *et al*., 2014; Pagano *et al*., 2016). According to Onaga *et al*. (2016), the use of nanofertilizer helps many plants to survive poor environmental circumstances, According to Wang *et al*. (2015), the use of nano ZnO is linked to increased proline and other amino acid accumulation, improved water deficiency and the activation of antioxidant enzymes. Nanoparticles are effective in detoxifying or remediating harmful pollutants like heavy metals. For example, Wang *et al*., (2015) showed that by controlling Cd accumulation, foliar application of nano-Si at a dose of 2.5 mM considerably improves rice plants' ability to withstand Cd stress. According to several studies (Wang *et al*., 2016; Shang *et al*., 2019), nano-Si fertilisers may putatively offer an advantage over standard fertilisers in reducing burdensomely heavy metal buildup. The two abiotic factors that affect plants the most are salt and drought (Phenny, 2018; Zhao *et al*., 2020). Intriguingly, Hojjat and Kamyab (2017) discovered that the effects of salt stress on fenugreek (*Trigonella foenum-graecum*) seed germination and growth were reversed by the administration of Ag-NPs. Due to promising results in this area, this nanobiotechnology application has recently seen enormous upheaval (Saeed *et al*., 2021). In order to control the activity of antioxidant enzymes such chloramphenicol acetyltransferase (FELINE), super oxide dismutase (SOD), and peroxidase, which are extremely effective in overcoming drought conditions, nanoparticles are used (Phenny, 2018). PerOxiDase (POD) activity, as well as that of Chl a and carotenoid content, dramatically decreased in 50 mg L $^{1}$ ZnO NPs (25 nm) and in 44 mg L $^{1}$ ZnSO4 despite a notable rise in SOD and CATalase (FELINE) activities being visually seen (Morales *et al.* 2013; Hu *et al.*, 2014). Numerous studies have been conducted on the function of nanoparticles in promoting plant development and resistance to metal stress (Asgari *et al.*, 2018; Hussain *et al.*, 2019). Si NPs may improve the development of plants exposed to metal stress by altering their nutritional status, photosynthesis, morphology, and physiology (Hussain *et al.*, 2019). In addition, CeO2 NP treatment increased the activity of N absorption enzymes such glutamine synthetase (GS), glutamine oxoglutarate aminotransferase, and glutamate dehydrogenase (GDH), which explains why plants with low nitrogen levels had high levels of N. To reduce N buildup under high nitrogen stress, CeO2 NPs, on the other hand, downregulated the GS and GDH activities (Bowne *et al.*, 2012).

## Nano Fertilizers and plant responses

Nanoparticles enter the body through natural openings such as stomata, hydathodes, stigmas and wounds (Sanzari *et al.*, 2019). Graphene eventually enhances crop amplitude while having no negative environmental effect (Kabiri *et al.*, 2017). Global nanotechnology market reached a value of 1 trillion dollars in 2015. Nanotechnology appears to be the alternative that might change this sector of agriculture. (Harper *et al.*, 2015). According to Xiong *et al.* (2017), copper oxide nanoparticles are used in agriculture as additives for soil remediation, plant growth regulators, insecticides, herbicides and fertilisers. CuO NPs buildup on lettuce and cabbage at concentrations up to 250 mg/L results in shortened water content and stunted vegetable development. They are leafy vegetables, which makes them vulnerable to air pollution in addition to absorbing CuO NPs from the soil. (Xiong *et al.*, 2017). It has been found that 0.3 mg/L Cu$^{2}$ surrendered from 1000 mg/L of copper nanoparticles enhances plant development and is not hazardous to the plant (Pestovsk *et al.*, 2017), but the absorbed quantities also rely on the kind of plant, the soil, and environmental conditions.

## Uptake, translocation and internalization of nanoparticles in plants

To learn more about the interaction between nanoparticles and plant surfaces, physiological and anatomical research on plants is necessary. Cuticles impede the majority of nanoparticles. As opposed to this, a study by Sanzari *et al.*, (2019) shown that $TiO_2$ nanoparticles are capable of creating an opening in the cuticle layer. Distribution strategies are essential for the internalisation of the nanoparticles into the plant. In watermelon, adding nanoparticles to the aerosol increased the rate of internalisation (Raliya *et al.*, 2016). Mucilage and exudates released by plant roots in the rhizosphere area attach nanoparticles to the surface of the roots and increase the rate of internalisation (Avellan *et al.*, 2017). Carbon nanotubes and nanoparticles with a diameter of 5 to 50 nm have been shown to effectively pierce the cell walls of many different plant species (Etxeberria *et al.*, 2016; Chang *et al.*, 2013). Apoplastic translocation promotes nanoparticle upward kineticism into the aerial region of the plants and radial kineticism of nanoparticles into the root vascular tissue (Sanzari *et al.*, 2019). The nanoparticle was found in the cytoplasm and nucleus of the endodermal cells in ryegrass, which is an apoplastic pathway of translocation, as shown by TEM pictures (Chandra *et al*., 2015). In rice, *Arabidopsis* and poplar plants, various-sized nanoparticles are transported through the plasmodesmata. However, $CeO_2$ nanoparticles applied to maize leaves did not successfully translocate into the plant's developing leaves (Birbaum *et al*., 2010). *Cucurbita maxima* can translocate $Fe_3O_4$ nanoparticles but *Phaseolus limensis* is unable to do so. Cu nanoparticles can be efficiently absorbed and transported by mungbean and wheat (Lee *et al*., 2008; Zhu *et al*., 2008). $TiO_2$ NPs applied to *Zea mays* in the studies of Asli and Neumann (Asli *et al*., 2009) were not translocated because their diameters exceeded the pore diameter (6.6 nm) of the root cells. Some of the nanoparticles in different research by Du *et al*. (2011) on the perforation of $TiO_2$ NPs in wheat plant went through the root cells whereas others did not. $CeO_2$ NPs significantly improved the photosynthetic rate of soybean under high soil moisture levels but not when soil water was restricted. Drought causes the plant's stomata to shut, which prevents transpiration and the absorption of $CO_2$ by the plant (Cao *et al*., 2018). Fullerol, which is said to be hydrophilic and smaller in size, may be transported into plant tissues by apoplastic channels (Lahiani *et al* 2013; Khodakovskaya *et al*., 2013). However, fullerenes and MWCNTs may easily interact with the hydrophobic elements of natural organic matter and may even penetrate the tissues of plants *Allium cepa* cell wall pores are reported to be blocked by them, making their translocation pointless (Hyung *et al*., 2007; Rico *et al*., 2011). In general, carbonaceous elements penetrate the tissues of plants (Chen *et al*., 2011; De La *et al*., 2016). A useful model that explains the many mechanisms for NPs absorption, translocation and sodality has been offered by Dietz and Herth (2011). Xylem diameter also affects how quickly water is transported, it is possible that this parameter will play a crucial role in the transport of ENMs in plant systems (Ma *et al*., 2010). Hischemoller *et al*., (2009) reported that axial transport occurs, which permits the penetration of the NPs solution into the velamen radicum after a few seconds of exposure, by tracking luminous NaYbEr4Yb,Er NanoCrystals (NCrs).

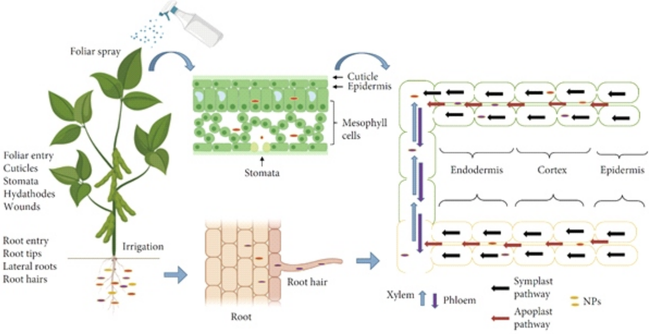

**Fig 2: Schematic presentation of nanoparticle uptake through different routes and their translocation pathways in different plants' parts (adapted from Ali *et al*., 2021)**

The absence of exodermis tissue in lateral roots allows NPs to enter the central cylinder and xylem in aqueous solution (Schreiber, 2010; Dietz and Herth, 2011). Fullerene C70 may easily be absorbed by roots and transferred to shoots, as well as entering plants by leaf absorption and travelling downward to the roots through the phloem, according to research on rice plants (*Oryza sativa*) (Yadav *et al*., 2014; Lin *et al*., 2009b). The soil-root channel appears to be the plant's primary route for absorbing CBNM. (Avanasi *et al*., 2014) showed that total fullerene C60 plant uptake was generally low (7%), with most of the accumulation taking place in the roots (40-47%) and more negligible amounts of accumulation taking place in the tuber (22-23%), stem (12-16%) and leaves (18-22%). This suggests that CBNMs released to the environment will not be highly bioavailable but will likely persist in soil (Liu *et al*., 2010).

## Nanoparticles in hydroponics

Hydroponics is the term for plant growth without soil. This method is often used to cultivate agricultural plants (Seaman and Bricklebank, 2011). A few different uses include crop development for food and biofuels. By growing metal nanoparticles in various plants, researchers have created hydroponics in nanotechnology (Schwabe *et al.*, 2013). Size and weight had a clear negative association with the concentration of CuO NPs (Shaw and Hossain, 2013). *Cucumis sativus* seedlings were treated to bulk copper oxide (CuO) and CuO nanoparticles at concentrations ranging from 100 to 600 mg L $^{1}$ in hydroponic research. Compared to control and bulk CuO, seed germination was reduced by 23.3% in the 600 mg L $^{1}$ CuO NPs treatment. Root elongation was reduced by CuO NPs by 50 and 34.2% at 100 and 600 mg/ L $^{1}$, respectively (Moon *et al.*, 2014). In hydroponics tests, the water-soluble fullerene C70(C(COOH)$_2$)$_4$ 8 inhibited the development of Arabidopsis, resulting in slowed roots with reduced length and a lack of root gravitropism. This research revealed problems in the cytoskeleton, eccentric cell division, root tips, and mitochondrial function (Liu *et al.*, 2010). In hydroponic cultures, $CeO_2$ NPs have been shown to interact with nutrients and organic materials (Van Hoecke *et al.*, 2009; Schwabe *et al.*, 2013).

## Nanoparticles improving biochemical responses in plants

Fe-NPs serve as an example of the NPs released by metallurgical industries. These NPs were used by (Canivet *et al.* 2015) in foliar exposure on *Physcomitrella patens*, assessing the effects on OS and membrane lipid peroxidation. ATP was measured during integration to assess the health of the plants. The sample accumulation was done over a period of 1 to 7 days, and the concentrations used varied from 5 ng to 50 mg. There have been reports of effects on a variety of factors, including growth, physiological and biochemical features, engenderment and food quality. But a thorough comprehension of the dynamics of interactions between plants and ENMs is still lacking (Zuverza-Mena *et al.*, 2017). Recent systems biology research supported the idea that metal nanoparticles (MNPs) cause a generalised stress replication with a high prevalence of oxidative stress components. The research included omics data from tobacco, rice, rocket salad, wheat and kidney beans (Ruotolo *et al.*, 2018). Metallic nanoparticles based on Iron oxide, ZnO, $TiO_2$, and copper have been directly irrigated into soil as nanofertilizers or applied topically to a variety of plants, including rapeseed, cucumber and mung bean plants (Gao *et al.*, 2006; Tarafdar *et al.*, 2014; Saharan *et al.*, 2016; Verma *et al.*, 2018). Similar to this, tomato plants exposed to MWNTs as soil supplements produced twice as many flowers and fruits, most likely due to the activation of genes and proteins critical for plant growth and development (Khodakovskaya *et al.*, 2013).

## Nanoparticles regulating antioxidant mechanism

Variations in each plant's physiological properties (EAs of RG, SOD, FELINE, and POD) rely on the kind and concentration of treatment (MO NPs), as well as specific drought stress circumstances. The forb *S. officinalis* and the grass *A. cristatum*, which were optically surveyed, showed the largest and lowest EA changes, respectively. Generally speaking, at lower MO NP concentrations (60 mg l $^{1}$), favourable effects (EA abbreviation) were visually assessed and led to plant resistance to drought stress levels. Application of MO NPs also maintains the RWC of plant tissues under Drough Stress (DS). AgO, FeO, ZnO, and CdO NPs at low concentrations (30–60 mg l $^{1}$) increased RWC by up to 11%. (Jahantab *et al.*, 2021). Plants integrate detoxifying systems by activating antioxidant enzymes (Shaw *et al.*, 2014). For instance, Trujillo-Reyes *et al.*, (2014) discovered that in lettuce roots exposed to Cu/CuO NPs, catalase (FELINE) activity increased but ascorbate peroxidase (APX) activity decreased.

## Nanoparticles regulating proteins and carbohydrates

Application of nano $Fe_3O_4$ to *Ocimum basilicum* improved essential oils, protein, chlorophyll and carbohydrates (El-Feky *et al.*, 2013). Between days 5 and 30, when plants were cultivated in a hydroponics environment and subjected to 10 mg $L^{1}$ of either AgNPs or $AgNO_3$, (Krishnaraj *et al.*, 2012) examined protein engenderment in the roots, stems and leaves of *Bacopa monnieri* (Linn) Wettst. Regardless of the treatment, protein concentration decreased similarly across the board in each plant component examined by day 10 (by nearly 50%) compared to day 5, moreover, an adjustment in protein synthesis was seen between days 20 and 30 of exposure. In general, protein levels in control plants were greater than those in the Ag treatments (Suresh *et al.*, 2016). In the leaves of plants treated with both materials, there was unmistakably an increase in glycoprotein, beta-sheet, and beta-turn protein structure in comparison to controls; nevertheless, it seems that nano-$Fe_2O_3$ had the greatest values. According to Koul *et al.* (2018), Au NPs had a significant impact on the expression levels of microRNAs, which regulated a variety of morphological, physiological and metabolic processes in plants. The gene profiles of Arabidopsis were examined by Wang *et al.* (2016) in replication to ZnO NPs and $ZnSO_4$. Geranyl pyrophosphate synthase (GGPS6), phytoene synthase (PSY), phytoene desaturase (PDS) and zeta-carotene desaturase were among the genes whose expression dramatically increased (ZDS). Lahiani *et al.*, (2013) demonstrated that MWCNTs activate seed germination and growth in soybean, maize, and barley seed coats (SIPs). On the other hand, single-walled carbon nanotubes (SWCNHs) can affect the expression of genes linked to cell development and stress signalling in tobacco plants. According to Rahman and Padavettan (2012), the main impact of nanoparticles on cells is the induction of reactive oxygen species (ROS) that result in significant cellular damages including mitochondrial, and plastid damage, which ultimately leading to cell death.

## Conclusion

Practises that encourage crop growth and output, such as excessive fertilisation, the use of artificial plant growth boosters and other inefficient resource management. Thus, it has been shown that using various metallic NPs increases redox potential, proline and chlorophyll levels and improves photosynthesis, stress tolerance and mineral intake. It may be possible to alter various physiological processes, including germination, fruit ripening and replication in stress conditions (both biotic and abiotic), where NO has been known to be implicated, by using NPs to transfer NO to specific plant targets. However, due to their similarity to heavy, ponderous metals, it is crucial to take into account the sinking of NPs into the soil that may have a harmful influence on plants.

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**RESEARCH ARTICLE**

# Impact of Dust on Plants affected by the Magnesite mining in the Kumaun region of lesser Himalayas

**Ashok Kumar Raghav**

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## Abstract

The research delved into the effects of dust on two prominent plant species, *Quercus leucotrichophora* and *Pinus roxburghii*, which were observed in the vicinity of magnesite mines. The study took place within the geographical coordinates of approximately 29° 45’30”N to 29° 47’30”N latitude and 79° 44’E to 79° 46’E longitude. Upon analysing the dust’s impact on vegetation, it became evident that there was a reduction in chlorophyll levels in the studied plant species, particularly in their early stages of exposure. Additionally, some variations were observed in the Chlorophyll a (chl a) to chlorophyll b (chl b) ratio. Notably, the thin layer of Magnesite dust covering the soil indirectly influenced vegetation by diminishing soil permeability and elevating alkalinity levels. Consequently, the physicochemical properties of the soil underwent significant alterations.

**Keywords:** Chlorophyll, Photosynthesis, Suspended Particulate Matter, Dust Particles, Ambient Air, $SO_2$, Soil and Magnesite

## Introduction

The escalation in quarrying, open-cast mining and road traffic has given rise to increased dust deposition on vegetation, impacting vital processes like photosynthesis, respiration and transpiration. This influx of dust also permits the penetration of phytotoxic gaseous pollutants. Consequently, visible signs of injury may manifest and overall productivity tends to decline.

Dust particles constitute a substantial portion of airborne pollutants generated by industrial activities, posing a grave threat to ecosystems. In India, approximately 30-35% of air pollutants consist of dust particles. Activities such as coal mining, quarrying, stone crushing, thermal power plants and cement industries release substantial quantities of dust into the environment. The current widespread deforestation, loss of biota and harm to other ecosystem elements can be attributed to the adverse effects of dust pollution.

Aerosols, due to their minute size, remain suspended in the air and readily adhere to solid surfaces they encounter. When air currents pass through trees, some dust particles stick themselves to the upper and lower leaf surfaces, while others are either deflected or deposited elsewhere based on particle size, wind speed and deposition surface area. Following deposition, dust particles tend to attach to leaf surfaces until they are washed away by rain or shredding of the leaves themselves.

Particles smaller than the the diameter of stomatal apertures can directly enter the sub-stomatal chamber and contact the spongy parenchyma within leaf tissue, while larger particles gain access to the leaf interior by dissolving in water and carbonic acid discharged by the stomata. Plants capture both dust and topsoil and microorganisms within the soil contribute to reducing air pollutants while breaking down or degrading various harmful chemicals that enter the.

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## Material and Methods

### Determination of Chlorophyll Content

100 mg of fresh leaf sample was ground in a motar-pestle with 80% cold acetone. A pinch of $CaCO_3$, was added to prevent chlorophyll degradation during extraction to ensure completeness of extraction. The extract was centrifuged at 5000 rpm for 15 minutes. The collected supernatant was diluted upto required constant volume. The samples were kept in dark / low temperature to avoid chlorophyll degradation. The optical density (O.D.) of the extract was measured at 663 and 645 nm wavelengths using a Spectronic 1201 spectrophotometer. The contents of chlorophyll a and b were calculated with the help of equation given by Maclachlan and Zalik (1963).

The plant species obtained from sites near the magnesite factory and mines were mainly species of Oaks (*Quercus leucotrichophora, Quercus himalayana, Quercus glauca, Quercus incana*). Other plant species include *Albizzia procera, Rhododendron arboretum, Myrica esculenta, Alnustunisia, Pinus roxburghii* etc.

The approximate densities of these plants around the study area were as follows:

**Table 1. Density of plant species**

| Species | Density (Plant.ha $^{1}$) |
|---|---|
| *Quercus leucotrichophora* | 360 |
| *Pinus roxburghii* | 330 |
| *Myrica esculent* | 140 |
| *Rhododendron arboretum* | 125 |
| *Quercus himalayana* | 120 |
| *Quercus glauca* | 20 |

Source: Almora Magnesite Limited, Almora

It was recorded that *Quercus leucotrichophora* and *Pinus roxburghii* were the most common and significant tree species in the area. The herbaceous species or shrubs were not taken into account for study because they were mostly annuals or biennials. The study of pinus trees was also ruled out because the needle-shaped leaves of plant hardly allow much dust to settle down. *Quercus leucotrichophora* was considered for observation in this Himalayan region because this species, besides providing fodder to the animals, also subscribe to a great deal to the water retention in the soil. The natural water springs are a common sight in the area dominated by oak trees. In the present study, *Quercus leucotrichophora* was chosen for studying chlorosis i.e. depletion in the level of chlorophyll. The location of sampling sites selected for the evaluation of the impact of dust on plants is shown in Fig.1.

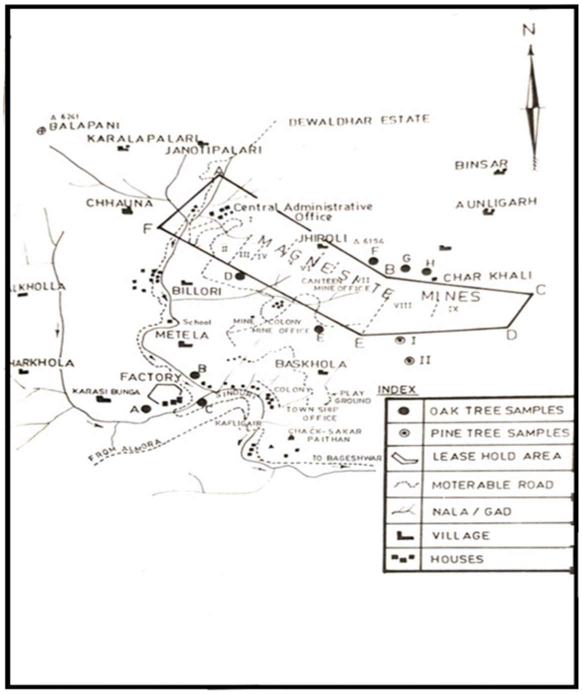

**Fig. 1:** Plant sampling sites for impact of dust

**Table 2. Description of sampling sites**

| S.No. | Sampling site | Distance from Plant/ Mines | Source |
|---|---|---|---|
| 1 | A | 1.50 Km | Plant |
| 2 | B | 2.50 Km | Plant |
| 3 | C | 2.00 Km | Plant |
| 4 | D | 200 meter | Mines |
| 5 | E | 400 meter | Mines |
| 6 | F | 215 meter | Mines |
| 7 | G | 225 meter | Mines |
| 8 | H | 250 meter | Mines |
| 9 | I | 300 meter | Mines |
| 10 | II | 1.5 Km | Mines |

**Samples A to H:** *Quercus leucotrichophora*

**Samples I and II:** *Pinus roxburghii*

Table 3. Observations

| S. No. | Sample | Chl a | Chl b | Chl a + Chl b | Chl a : Chl b |
|---|---|---|---|---|---|
| 1 | A | 0.525 | 0.290 | 0.815 | 1.810 |
| 2 | B | 0.637 | 0.502 | 1.139 | 1.260 |
| 3 | C | 0.597 | 0.526 | 1.123 | 1.130 |
| 4 | D | 0.252 | 0.205 | 0.455 | 1.220 |
| 5 | E | 0.575 | 0.598 | 1.173 | 0.960 |
| 6 | F | 0.415 | 0.397 | 0.812 | 1.045 |
| 7 | G | 0.421 | 0.467 | 0.888 | 0.901 |
| 8 | H | 0.429 | 0.385 | 0.814 | 1.110 |
| 9 | I | 0.727 | 0.635 | 1.362 | 1.140 |
| 10 | II | 0.747 | 0.658 | 1.405 | 1.130 |

Samples A to H: *Quercus leucotrichophora*
Samples I to II: *Pinus roxburghii*

## Results

In the present investigation, chlorophyll a was found to be lowest in sample D which is nearest to the mining area, while it was recorded to be highest in sample B which is farthest from the factory source. The chlorophyll b was reported to be lowest in sample D and highest in sample E. For the ratio of chlorophyll a and b, the lowest values were observed in sample E. and highest values in sample A. The observations clearly revealed that chlorophyll level varies with distance from the source. Nearer is the site to the source, lower is the level of chlorophyll. However, samples F, G, H though being nearer to the source showed a significantly high level of chlorophyll, the higher content of chlorophyll at these sites can be ascribed to their position at higher altitudes where particulate matter and gaseous substances get adsorbed on the dust and do not settle down quickly.

In this study, Pine trees do not manifest any change in the chlorophyll contents. The samples collected from the site nearest to the source (sample Pinus I) and the sample collected from the site away from the source (sample Pinus II) gave similar values for chlorophyll a and b and chlorophyll a and b ratio for that matter.

## Discussion

The effect of dust on plant communities can broadly be classified into two categories:

### Direct effects

These are caused by the contact between the dust particles and the plant tissues.

### Indirect effects

These are caused by the alteration in physicochemical properties of the soil which in turn affects plant growth.

### Direct Effects

Since some variations in the concentration of chlorophyll a and b and the ratio of chl a and b have been observed, it can be concluded that impact of air pollution as a result of dust on the most prominent flora around the study site exists but it is in initial stages.

In the present study, no visible foliar injury on plants has been observed, because of the low concentration of $SO_2$ in the ambient air. But the diminution of chlorophyll in plant species under observation indicates the effect of air pollution in initial or preliminary stages.

Degradation of photosynthetic pigment has widely been used as an indication of pollution (Le Blanc and Rao, 1973; Schulz, 1986; Silberstein and Galum, 1988; Singh *et al.* 1990). Chlorophyll, the green pigment is one of the main complexes (Plastid and Proteins) which influences photosynthesis. The degradation of chlorophyll pigments can be attributed to the phytotoxic action of $SO_2$ (Lauenroth and Dodd, 1981). Rao and Le Blanc (1965) observed the destruction of chlorophyll molecules in lichens following exposure to large doses of gaseous $SO_2$. They described that in this process $Mg^2$ of the chlorophyll molecule is replaced by two atoms of hydrogen forming phaeophytin. Sharma and Butler (1975) and Gupta and Ghouse (1986) recorded low stomatal frequency, higher trichome density and length in plants of $SO_2$ polluted areas. Reports of decreased chlorophyll concentration in plants of $SO_2$ are often associated with degradation of chlorophyll a (Bell, 1980; Ayer and Bedi, 1986, Kumar, 1986), However, instances of simultaneous destruction of chl a and chl b are also known (Singh *et al.*, 1988; Khan *et al.*, 1990; Singh *et al.*, 1990). According to Krishnamurthy and Raja Chidambaram (1986), a 1 mm thick coating of dust on the lamina could prevent the entry of as much as 90% of radiant energy incident on the leaf which resulted in hampering the chlorophyll biosynthesis.

Chl a decomposes more rapidly than chlorophyll b (Chl b). At the high concentration of $SO_2$, there is a change in the ratio of Chl a and Chl b, so also the formation of phaeophytin. But at low concentrations of $SO_2$, Chl a and Chl b do not decompose rapidly and the change in the ratio of Chl a and Chl b is not significant.

Pierce (1909, 1910) and Parish (1910) observed that settled dust in combination with mist or light rain formed a relatively thick crust on the upper leaf surfaces. Pierce (1910) demonstrated that encrustation of cement kiln dust on citrus leaves interfered with light required for photosynthesis, resulting in reduced starch formation. This was later confirmed by Czaja (1962) and Bohne (1963) in a number of plant species.

Effect of fly ash on chlorophyll content has been observed by many workers (Vaccarino *et al.*, 1983; Mishra and Shukla, 1986; Ahmad *et al.*, 1987). At high application of fly-ash, photosynthetic pigment decreases. Reduction of chlorophyll content at high dusting rates may be due to alkalinity caused by excessive soluble salts on the leaf surfaces. Chlorophyll degradation is also possible because of thick fly ash covering over the surface of the leaf, which inhibits transpiration and eventually evaporative cooling. The reduction in chlorophyll pigment in leaves thickly covered with fly ash has been reported to increase foliar temperature which retards chlorophyll synthesis (Garg and Varshney, 1985). Rao (1985) also reported chlorophyll destruction and foliar necrosis in coal dust affected mango and lemon plants.

## Indirect Effects

Indirect effects on the plant growth are caused by the toxic dust generated from Dead Burnt magnesite plant and mining area which are rich in silica and MgO. This dust creates a crust of thin layer over soil (Fig.1.2) In the presence of moisture, this crust creates anaerobic conditions and changes in the physicochemical properties of the soil. Bermadinger *et al.* (1988) reported that the dust containing MgO generated from Magnesite factory in Austria has influenced the wax tubes of plant leaves in a more serious way, the wax tube fuse and the crystalline structure is lost. Stratman and Van Haunt (1956) stated that dust falling on the soil caused a shift in pH to the alkaline side, which was unfavourable to oats, but favourable to pasture grass.

**Fig. 2:** Coating of Magnesite dust on the land of village Sinduri

## Conclusion

The examination of dust's influence on vegetation has unveiled a decrease in chlorophyll levels within the studied plant species, primarily at the early stages of observation. Furthermore, there were notable variations in the ratio of chlorophyll a to chlorophyll b. It is also worth noting that the thin layer of magnesite dust on the soil indirectly impacts vegetation by reducing soil permeability and elevating alkalinity levels. This, in turn, triggers a transformation in the physiochemical properties of the soil.

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**RESEARCH ARTICLE**

# Utilizing Statistical Tools in Genetic Studies: Applications, Challenges, and Future Directions

**Ajay Kumar Agarwal**

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## Abstract

Genetic studies have witnessed a paradigm shift with the advent of high-throughput technologies, enabling researchers to generate massive datasets for understanding the complexities of the human genome and other organisms. Statistical tools play a pivotal role in extracting meaningful insights from these vast datasets, aiding in the identification of genetic variations, association studies, and unraveling the genetic basis of complex traits. This research paper provides a comprehensive review of the applications of statistical tools in genetic studies, discusses the challenges associated with their implementation, and outlines potential future directions in this rapidly evolving field.

**Keywords:** Statistical genetics, Genome-wide association studies (GWAS), Polygenic risk scores (PRS)

## Introduction

The field of genetic studies has undergone a transformative evolution, catalyzed by the unprecedented growth of high-throughput sequencing technologies and the generation of vast genomic datasets. The deciphering of the human genome through the Human Genome Project marked a historic milestone, laying the foundation for unraveling the intricate genetic code underlying biological processes and disease susceptibility. However, the translation of genomic information into meaningful insights requires sophisticated analytical approaches, and statistical tools have emerged as indispensable instruments in this genomic era.

Understanding the genetic basis of complex traits and diseases demands the identification and interpretation of genetic variations across populations. Statistical methodologies play a pivotal role in genetic studies by providing the tools and techniques necessary to analyze, interpret, and draw meaningful conclusions from complex genetic data ( D'Agostino, et al 2003). The integration of statistical methods in genetic research enables scientists to unravel the genetic basis of various traits, diseases, and population-level variations. Statistical methodologies have become paramount in the dissection of these variations, enabling researchers to conduct large-scale investigations with a level of detail and precision previously unattainable. This paper aims to delve into the multifaceted applications of statistical tools in genetic studies, examining their pivotal role in unraveling the intricacies of the genome (Almgren, *et al* 2003).

The paper discusses the role of statistical tools in functional annotation and variant prioritization. As genetic datasets continue to expand, the ability to discern the functional implications of genetic variations becomes increasingly crucial. The integration of statistical methodologies in annotating functional elements and prioritizing variants enhances the interpretability of genomic information, contributing to our understanding of the biological consequences of genetic variation. The integration of statistical tools in

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genetic studies represents a cornerstone in the quest to unravel the mysteries of the genome (Montana, 2006). Through their applications in GWAS, linkage analysis, functional annotation, and beyond, these tools provide the means to extract meaningful insights from the vast troves of genomic data. As we stand at the precipice of unprecedented technological advancements, this paper will also outline the future directions in which statistical methodologies are likely to evolve, opening new frontiers in our quest to decode the genetic basis of life and disease.

## Different statistical methodologies used in genetic studies:

1. **Data Exploration and Descriptive Statistics:** Descriptive statistics are brief informational coefficients that summarize a given data set, which can be either a representation of the entire population or a sample of a population. Descriptive statistics for continuous variables fall into 3 general classes, viz: location statistics (eg, mean, median, mode, quantiles), dispersion statistics (eg, variance, standard deviation, range, interquartile range), and shape statistics (eg, skewness, kurtosis). Statistical methods employed for Measures of discriptive statistics, providing a concise overview of the central tendency and variability of genetic data. Measures of central tendency describe the centres of the data set and measures of variability describe the dispersion of the data set . In short descriptive statistics helps to describe and understand the features of a specific data set by giving short summaries about the sample and measures of the data.

   Descriptive statistics play a fundamental role in genetic studies by summarizing and characterizing the features of genetic data. They facilitate the interpretation of genetic variation, help identify patterns, guide quality control processes, and contribute to effective communication of findings within the scientific community and beyond.

2. **Inferential Statistics:** Inferential statistics are mathematical calculations performed to determine if the results from the sample data are likely due to chance or are a true representation of the population. Inferential statistics are used to determine the probability of chance alone leading to sampled results. Statistical tests are used to evaluate hypotheses about the relationships between genetic variables and phenotypic traits (Pierce, 2005). Common tests include t-tests, chi-square tests, and analysis of variance (ANOVA). Statistical methodologies provide techniques to estimate confidence intervals, which quantify the uncertainty around parameter estimates and aid in the interpretation of genetic associations. All inferential statistical tests end with a test statistic and the associated P value. The p-value is based on two hypotheses. One of these is the null hypothesis, in which it is normally assumed that there is no difference or no effect of a treatment . The second and alternative hypothesis is often an assumption that the null hypothesis is untrue. Then a set of mathematical-statistical methods are applied to estimate the probability of what we are observing, given that the null hypothesis is actually correct (Fisher, 1925). The probability of what we are observing or any greater deviations from the null hypothesis – assuming that the null hypothesis is correct – is our p-value.

3. **Regression Models:** Regression analysis plays a crucial role in genetic studies by helping researchers understand the relationship between genetic factors and observable traits or outcomes. Genetic studies aim to unravel the influence of genes on various phenotypes, such as diseases, traits, or behaviors. Regression analysis is a statistical method that allows researchers to model and quantify the association between genetic variables and phenotypic outcomes while controlling for other relevant factors. Regression analysis is employed in various genetic studies, that allows researchers to identify genetic variants, quantify their effects, control for confounding factors, and gain insights into the genetic basis of various traits and diseases.

The field of genetic studies relies heavily on advanced statistical tools to extract meaningful insights from complex genomic data. In genetic studies, mathematical tools might include statistical methods, algorithms for data imputation, equations for linkage analysis, and other quantitative approaches applied to genomic data. These tools, grounded in mathematical formulations, enable researchers to analyze and interpret genetic information, sorting out the intricate relationships between genetic variants and phenotypic traits. Therefore, we look into the mathematical foundations of key statistical tools commonly employed in genetic studies.

1. **Genome-Wide Association Studies (GWAS)**

   Genome-wide association studies hinge on logistic regression models to explore the relationship between genetic variants and binary outcomes, such as the presence or absence of a trait (Uffelmann et al. ,2021). The fundamental equation, logit $(P(Y=1))=\hat{a}_0+\hat{a}_1X_1+\hat{a}_2X_2+\ldots+\hat{a}_nX_n$ ?, captures the essence of this statistical approach, where Y represents the binary outcome, $X_i$? denotes genetic variants, and $\hat{a}_i$? signifies the regression coefficients.

2. **Linkage Analysis**

   Linkage analysis, a fundamental tool for identifying genetic markers linked to specific traits, involves the calculation of LOD scores. The mathematical equation - LOD = $\log_{10}$ (Likelihood under/ linkage/ Likelihood under no linkage) quantifies the strength of evidence for linkage, providing researchers with a robust statistical measure to assess genetic associations.

3. **Haplotypes and Linkage Disequilibrium**

   In the realm of haplotype analysis and linkage disequilibrium studies (Niu, 2004), mathematical equations such as $D^2$ and $r^2$ provide insights into the relationships between alleles on the same chromosome.

   **Equations**: $D_2 \quad \frac{p_{12}q_{21} \quad p_{21}q_{12}}{\sqrt{p_1p_2q_1q_2}}$

   $$r^2 \quad \frac{(p_{12} \quad p_1p_2)(q_{12} \quad q_1q_2)}{\sqrt{p_1p_2q_1q_2}}$$

   Here, p1,p2,q1,q2? are allele frequencies, and p12,q12,p21,q21? are observed haplotype frequencies. These equations, involving allele frequencies and observed haplotype frequencies, quantify the extent of linkage disequilibrium and aid in the identification of haplotype blocks.

4. **Imputation and Phasing (BEAGLE)**

   BEAGLE, a tool for imputing missing genotypes and phasing haplotypes, relies on Hidden Markov Models (HMM). The mathematical formulation of HMM involves transition probabilities, emission probabilities, and the Viterbi algorithm, facilitating the efficient calculation of the most likely hidden states (haplotypes) given observed genotype data.

5. **Rare Variant Analysis (SKAT)**

   The Sequence Kernel Association Test (SKAT) addresses the challenges posed by rare variants through a score test statistic. The equation $Q_{SKAT}=(X\hat{a})^T V?1(X\hat{a})/Var(X\hat{a})$ ? encapsulates the essence of SKAT, where X represents the design matrix, â denotes the vector of effect sizes, and V is the variance-covariance matrix.

6. **Functional Annotation (VEP)**

   In functional genomics, Variant Effect Predictor (VEP) utilizes diverse mathematical algorithms to predict the functional consequences of genetic variants. These algorithms incorporate evolutionary conservation scores, structural information, and other features to quantify the potential impact of variants on gene function.

7. **Meta-analysis**

   Meta-analysis combines results from multiple genetic association studies using a weighted average of effect sizes. The equation ?? encapsulates this approach, where wi? represents the weight assigned to each study based on the inverse of the variance of the effect size estimate.

8. **Population Structure Correction (Eigenstrat)**

   Eigenstrat employs Principal Component Analysis (PCA) to correct for population substructure (Scheet and Stephens, 2006).. The mathematical formulation $X=U\acute{O}V^{T}$ involves eigenvector matrices (U,V) and a diagonal matrix of eigenvalues (Ó), capturing the major axes of genetic variation.

All the above mentioned the mathematical frameworks, equations, and algorithms that drive the analysis and interpretation of genetic data to provide a robust framework for researchers to navigate the complexities of genomic data.From logistic regression models in GWAS to LOD scores in linkage analysis and the intricacies of HMMs in imputation, these mathematical formulations empower geneticists to unlock the mysteries of the genome, fostering a deeper understanding of the genetic basis of traits and diseases.

## Applications of Statistical Tools in Genetic Studies

The application of statistical tools in genetic studies has become indispensable for extracting meaningful insights from the vast and complex genomic data generated in modern research. Statistical methods play a crucial role in identifying genetic variations associated with diseases, traits, and susceptibility, helping researchers unravel the underlying genetic architecture. As genomic datasets continue to expand, advanced statistical models, including machine learning algorithms, are employed for tasks such as fine-mapping causal variants, predicting gene expression, and integrating multi-omics data. Overall, the application of statistical tools not only facilitates the interpretation of genetic data but also contributes to the translation of genomic discoveries into actionable insights for healthcare and personalized medicine. Some of these are discussed below

### 1. Genome-Wide Association Studies (GWAS)

Genome-Wide Association Studies (GWAS) have emerged as a powerful approach for uncovering the genetic basis of complex traits and diseases. In these studies, researchers investigate the entire genome to identify common genetic variants associated with specific phenotypes (Uffelmann, *et al* 2021). The statistical methods employed in GWAS involve examining the relationship between genetic markers, typically single nucleotide polymorphisms (SNPs), and the trait of interest. Logistic regression or linear regression models are commonly used to assess the strength and significance of these associations. Notable successes include the identification of genetic variants linked to conditions such as diabetes, cardiovascular diseases, and various cancers. The comprehensive nature of GWAS enables researchers to explore the polygenic nature of many traits, providing valuable insights into the genetic architecture of complex phenotypes.

### 2. Linkage Analysis

Linkage analysis plays a crucial role in identifying genetic loci responsible for Mendelian traits within families. This method is particularly effective when studying rare variants with larger effects. Statistical methods in linkage analysis, such as the logarithm of odds (LOD) score, assess the likelihood of genetic linkage between markers and the trait of interest across multiple generations. Unlike GWAS, which focuses on common variants in populations, linkage analysis is well-suited for rare variants that may exhibit stronger effects within specific family pedigrees. By exploring the familial aggregation of genetic markers and traits, linkage analysis complements the findings of GWAS and provides a comprehensive understanding of the genetic basis of heritable conditions.

### 3. Functional Annotation and Variant Prioritization

As genomic sequencing technologies advance, the need to prioritize functionally relevant genetic variants becomes paramount.

Statistical tools are instrumental in functional annotation and variant prioritization, aiding researchers in identifying the most biologically meaningful variations. Computational models integrate information from diverse sources, including evolutionary conservation, protein structure, and functional genomics data. These models assign functional scores to variants, guiding researchers in selecting variants for further experimental validation. By incorporating statistical methodologies into the interpretation of genomic data, researchers can prioritize variants with potential functional consequences, facilitating the identification of genetic elements influencing diseases and traits.

### 4. Polygenic Risk Scores (PRS)

Polygenic Risk Scores (PRS) have emerged as predictive tools for assessing individual genetic susceptibility to complex diseases. In the development of PRS, statistical models integrate information from multiple genetic variants, each contributing a small effect to the overall risk prediction (Osterman, *et al* 2021). These scores provide a quantitative measure of an individual's genetic predisposition to a particular trait or disease. While PRS have demonstrated success in predicting disease risk, ongoing research is addressing challenges such as model overfitting, population-specific differences, and the ethical implications of using genetic information for risk assessment. The application of statistical tools in developing and refining PRS exemplifies their role in advancing personalized medicine and risk prediction based on an individual's genetic makeup.

### Challenges in Implementing Statistical Tools in Genetic Studies

Implementing statistical tools in genetic studies is a crucial yet challenging endeavor. The complexity of genomic data, the need for large-scale analyses, and the evolving nature of genetic research pose several challenges. The challenges in implementing statistical tools in genetic studies are multifaceted and deeply rooted in the unique characteristics of genetic data. The enormous size of datasets generated by high-throughput sequencing technologies poses computational challenges (Bogdan, et al, 2004) requiring scalable and efficient algorithms to process and analyze the huge amount of genetic information. Data quality and preprocessing are critical aspects, given the inherent noise in genetic data. Researchers must carefully filter out artifacts, correct biases, and ensure the integrity of the data without introducing unintended distortions.

Population structure adds another layer of complexity, particularly in studies involving diverse populations. Failure to account for population stratification may lead to erroneous associations, demanding the development of sophisticated statistical methods to discern genuine genetic signals from confounding factors. Identifying rare variants and deciphering polygenic traits, often characterized by the involvement of numerous genetic factors with small individual effects, present additional hurdles, necessitating advanced statistical models for accurate detection and interpretation.

Further, ensuring statistical power, especially in studies dealing with rare variants or small sample sizes, is paramount for detecting genuine genetic associations amidst the plethora of data. Addressing multiple testing issues, such as false positives arising from the sheer volume of statistical tests conducted, requires careful correction methods to strike a balance between sensitivity and specificity.

Interpretability and reproducibility are ongoing challenges in genetic studies. The complex nature of genetic findings demands a nuanced interpretation, and ensuring that results can be consistently replicated across different studies is essential for establishing the validity of genetic associations. Moreover, the dynamic nature of genomic data, marked by continuous technological advancements and the discovery of new genetic elements, necessitates the perpetual adaptation of statistical methods. Ethical considerations and privacy concerns further underscore the need for researchers to adhere to stringent guidelines, ensuring the responsible handling and secure storage of genetic data.

Lastly, the integration of multimodal data from various sources, such as genomics, transcriptomics, and epigenomics, introduces another layer of complexity. Developing statistical tools capable of effectively handling and extracting meaningful insights from these diverse datasets is imperative for a comprehensive understanding of the genetic landscape. Thus it can be concluded that navigating the challenges in implementing statistical tools in genetic studies requires a combination of computational innovation, methodological sophistication, and interdisciplinary collaboration. Overcoming these hurdles is essential for advancing our understanding of the genetic underpinnings of complex biological phenomena and translating this knowledge into meaningful applications for human health and medicine.

## Future Directions

The future of utilizing statistical tools in genetic studies is on the edge of remarkable advancements across various fronts. Integration of multi-omics data, encompassing genomics, transcriptomics, epigenomics, and proteomics, will be a cornerstone, necessitating the development of sophisticated statistical methods capable of unraveling the intricacies within these complex datasets. The increasing prevalence of machine learning and artificial intelligence will play a pivotal role, enabling pattern recognition and feature extraction in large-scale genetic data. Emphasis on diverse populations will drive the development of statistical approaches that account for population structure and genetic diversity. Polygenic risk scores (PRS) are poised to become integral, with ongoing efforts to enhance their accuracy and applicability in clinical settings. Causal inference methodologies, particularly Mendelian randomization, will see further refinement to establish causal relationships in genetic associations. As single-cell genomics gains prominence, statistical tools will evolve to analyze individual cell-level data, uncovering insights into cellular heterogeneity. The adaptability of statistical tools to real-time data analysis, especially with the rise of technologies like real-time DNA sequencing, is anticipated, promising a future where genetic studies are at the forefront of personalized and precision medicine. Some of these includes

1. **Machine Learning Approaches**

   The future of genetic studies lies at the intersection of genomics and machine learning. Machine learning approaches, with their capacity to handle complex, high-dimensional datasets, hold immense potential for uncovering novel patterns and associations within genomic data (Bogdan et al, 2004). Techniques such as deep learning, ensemble methods, and neural networks can enhance our ability to identify intricate genetic relationships and predict complex traits. Integrating machine learning into genetic research offers opportunities to unearth non-linear relationships, capture subtle interactions among genes, and improve the accuracy of predictive models. However, it is essential to address challenges such as interpretability, model generalizability, and the need for large, diverse training datasets to ensure the responsible and effective implementation of machine learning in genetic studies.

2. **Single-Cell Genomics and Spatial Transcriptomics**

   The advent of single-cell genomics and spatial transcriptomics represents a paradigm shift in understanding cellular heterogeneity and spatial organization within tissues. Future genetic studies are poised to incorporate these technologies to dissect complex biological systems at unprecedented resolution. Single-cell RNA sequencing enables the profiling of individual cells, offering insights into cell-type-specific gene expression patterns and cellular states. Spatial transcriptomics complements this by providing information on the spatial distribution of gene expression within tissues. Integrating statistical tools with single-cell and spatial omics data will unravel intricate cellular interactions, identify novel cell types, and enhance our understanding of tissue-specific genetic regulation. These advancements have significant implications for studying development, disease progression, and therapeutic interventions at a cellular and spatial level.

3. **Long-Read Sequencing and Structural Variants**

   The continued evolution of sequencing technologies towards long-read sequencing addresses the limitations of short-read sequencing, particularly in capturing complex structural variations in the genome. Future genetic studies will benefit from improved detection and characterization of structural variants, such as large insertions, deletions, and inversions. Statistical tools tailored for long-read data analysis will play a crucial role in identifying the functional consequences of structural variants and their contributions to diseases. Understanding the impact of structural variations on gene regulation, genomic stability, and disease susceptibility will provide a more comprehensive view of the genetic landscape. Integrating long-read sequencing data into genetic studies represents a critical step towards unraveling the full spectrum of genomic variations.

4. **Ethical Considerations and Privacy**

   As genetic studies progress, ethical considerations and privacy safeguards become increasingly crucial. Future directions in genetic research must prioritize the responsible use of genetic information, considering the potential implications for individuals and communities. Establishing clear guidelines for informed consent, data sharing, and protection of participant privacy will be paramount. Additionally, addressing issues of potential misuse, unintended consequences, and the societal impact of genetic research demands ongoing collaboration between researchers, ethicists, policymakers, and the public. Emphasizing transparency, inclusivity, and ethical governance will ensure that genetic studies continue to advance scientific knowledge while respecting the rights and autonomy of individuals whose genetic data contribute to these studies.

## Conclusion

In conclusion, the integration of statistical tools in genetic studies represents a dynamic frontier, propelling advancements in our understanding of the intricacies of the genome. The multifaceted applications of these tools, ranging from unraveling complex traits through GWAS to embracing cutting-edge technologies like single-cell genomics and long-read sequencing, underscore their pivotal role in shaping the future of genetic research. However, as we navigate this genomic era, vigilance toward ethical considerations and privacy safeguards is imperative. The responsible use of genetic information, transparency, and collaborative efforts will ensure that genetic studies continue to contribute meaningfully to scientific knowledge while upholding the principles of ethical research and respecting individual privacy. The future of genetic studies holds promise for transformative discoveries, with statistical methodologies guiding the way towards precision medicine and a deeper comprehension of the genetic foundations of life and disease.

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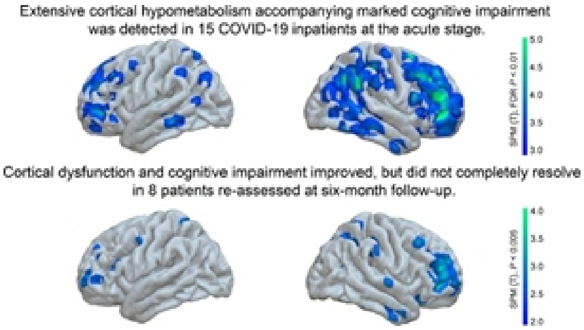
# RECENT NEWS

## COVID-19: Blood clot proteins linked to lingering cognitive deficits in severe cases

A recent study has revealed a potential link between blood clotting proteins and cognitive deficits in individuals who experienced severe COVID-19, particularly those with long COVID. The study focused on unvaccinated individuals hospitalized with COVID-19 early in the pandemic, analyzing data from over 1800 patients. It identified two blood clotting proteins, fibrinogen and D-dimer, whose elevated levels during hospitalization were associated with cognitive deficits up to 1-year post-infection. The study suggests that these proteins may play a role in developing long COVID symptoms, such as difficulty concentrating and memory issues, shedding light on a possible mechanism behind lingering cognitive problems in some COVID-19 survivors.



While the findings provide valuable insights into potential biomarkers for long COVID, the study has some limitations. It primarily examined unvaccinated individuals with severe COVID-19, raising questions about the generalizability of the results to the broader population, especially those with milder initial infections. Additionally, the study does not offer immediate diagnostic tools for long COVID but rather highlights fibrinogen and D-dimer as potential indicators of cognitive problems at 6 months or 12 months post-infection.

The study's results may contribute to ongoing research into the mechanisms of long COVID and stimulate further investigation into the role of blood clotting proteins and related biological pathways. However, the study does not provide conclusive evidence for specific treatments, and the complex nature of long COVID continues to pose challenges in diagnosis and intervention.

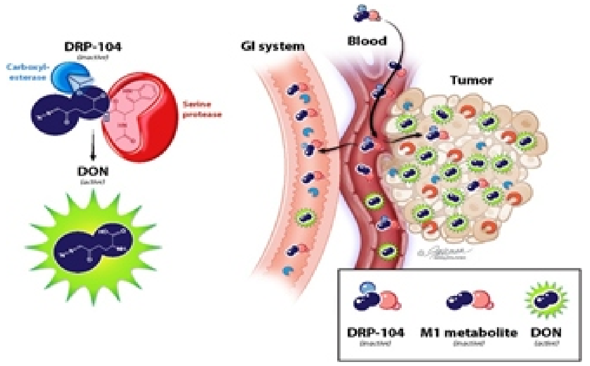
## Yeast speeds discovery of medicinal compounds in plants

Researchers have developed an innovative and cost-effective method utilizing baker's yeast to decipher how plants synthesize medicinal compounds. Traditional approaches involve analyzing plant transcriptomes, identifying numerous genes potentially coding for enzymes facilitating production. However, the biochemical characterization of each gene is a laborious process. The new yeast-based screening method captures protein-protein interactions between plant enzymes, complementing existing methods to pinpoint genes responsible for a plant's biosynthesis of medicinal compounds. This approach reduces the number of genes that need biochemical screening, making the discovery process more efficient and cost-effective.

The study demonstrated the yeast-based method by focusing on kratom leaves. Kratom, a tropical tree native to southeast Asia, has gained attention for its pharmaceutical potential, particularly the production of mitragynine, which exhibits painkilling effects without dangerous respiratory depression. The yeast-based method identified six kratom enzymes out of 20 predicted candidates, streamlining the discovery process and providing a more accurate and economical means of producing mitragynine and other potentially valuable chemicals. This research opens avenues for efficient drug discovery processes and continued exploration of the pharmaceutical potential of the kratom tree.

## New-found mechanism suggests drug combination could starve pancreatic cancer

A newly developed drug, DRP-104, designed to inhibit pancreatic ductal adenocarcinoma (PDAC) cells from switching fuel sources, is successfully contributing to the deadliness of pancreatic cancers. PDAC cells typically use the enzyme glutaminase to convert the amino acid glutamate into glutamine, a form burned for fuel to sustain rapid tumor growth. However, drugs designed to block glutaminase often lead cancer cells to switch to alternative pathways. DRP-104, currently in clinical trials for non-small cell lung cancer, cannot be burned as fuel but clings to the same enzymes as glutamine. When combined with an existing drug, trametinib, the treatment improved survival in mouse models of pancreatic cancer compared to DRP-104 alone.



The study, published in Nature Cancer, addresses the challenge of translating the understanding of how cancer cells switch fuel sources into clinically relevant therapies. DRP-104, a prodrug form of 6-Diazo-5-oxo-L-norleucine (DON), is designed to overcome toxicity issues associated with DON and mimic glutamine, broadly inhibiting all metabolic pathways that use glutamine. When combined with trametinib, which blocks the ERK signaling pathway, the treatment further improved survival in mouse models of pancreatic cancer. The researchers hope that this approach, already in clinical trials, may lead to improved patient outcomes for pancreatic cancer, a highly resistant form of cancer.

The success of such approaches depends on carefully balancing improved therapeutic efficacy and potential toxicity effects on normal tissues. The researchers plan to investigate how glutamine antagonism impacts other adaptive nutrient scavenging mechanisms in pancreatic cancers and whether these mechanisms could be targeted as well. The study was funded by grants from Perlmutter Cancer Center, National Cancer Institute, Lustgarten Foundation, Stand Up to Cancer, and the Howard Hughes Medical Gilliam Fellowships.

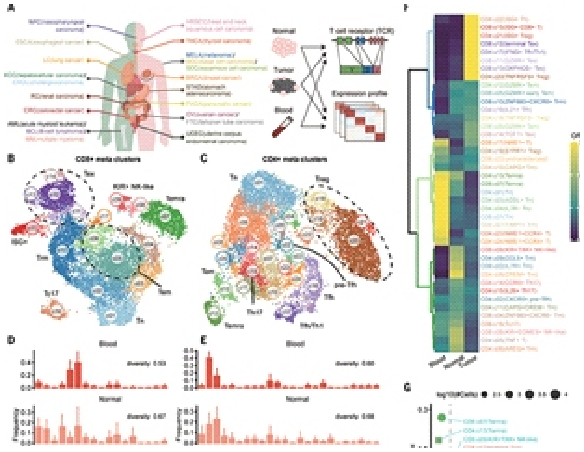
## The waxy surface protecting plants might hold the key to developing stronger crops

A recent study has discovered that cuticular waxes, a thin layer that plants deposit on their surface to protect against water loss, can break down to release compounds that play a role in plant signaling and communication. This finding challenges the traditional view that these waxes are stable and unreactive barriers. The study focused on the breakdown of unsaturated waxes, known as alkenes, in a species of poplar tree, revealing the production of aldehyde signaling compounds and insect pheromones. The researchers suggest that understanding this process could eventually be harnessed to develop stronger, more resilient plants, particularly in dealing with challenging environmental conditions such as drought or insect infestations.

The research published in the proceedings of the National Academy of Sciences, highlights the potential for engineering desirable traits in plants by leveraging the breakdown of waxes. Aldehydes, released during this process, play essential roles in both plant and animal systems, influencing growth, development, reproduction, and even serving as signaling molecules in plant-to-plant communication. The study's findings open up possibilities for using this knowledge to improve crop resilience, potentially developing natural alternatives for insect attraction or repulsion. The discovery challenges the traditional understanding of plant waxes as static barriers, offering a new perspective on the dynamic role these compounds play in the life cycle of plants and their interactions with the environment.

## Revolutionizing Immunotherapy: Scientists Map T Cell Blueprint with Next-Gen CRISPR

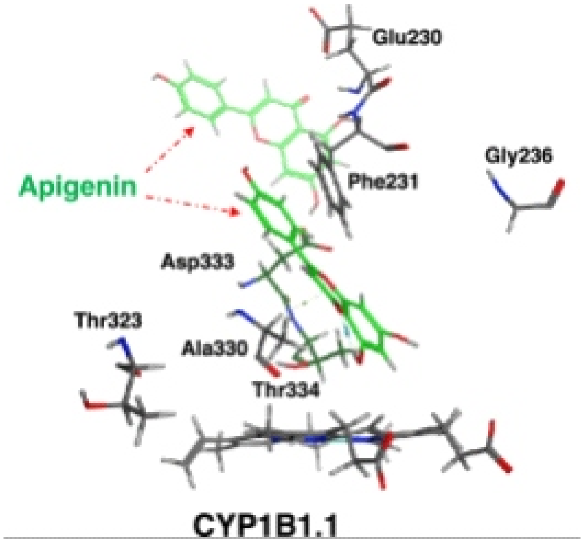
In a groundbreaking study, researchers at Gladstone Institutes have unleashed the power of next-generation CRISPR technology, known as base editing, to create a detailed molecular map of human T cells—the orchestrators of our immune system. By scrutinizing over 100,000 sites across nearly 400 genes, the team unveiled specific nucleotides influencing how T cells respond to stimuli. This intricate mapping not only promises to overcome current immunotherapy limitations but also holds the key to identifying novel drug targets for diseases ranging from cancer to autoimmune disorders. Dr. Marson, director of the Gladstone-UCSF Institute of Genomic Immunology, expresses excitement, stating that these "precise and informative maps" will shape the future of tailored immunotherapies, opening new frontiers in the battle against complex diseases.



In a bid to unravel the genetic basis of immune cell functions, the study marks a significant leap forward. Leveraging base editing, the scientists achieved high-resolution insights into T cell DNA, offering blueprints for drug development, diagnostics, and scientific exploration. As computational genomics takes center stage in processing the immense pool of data, the study's co-first author, Carl Ward, emphasizes the newfound ability to decode specific mutations, previously shrouded in mystery. Ward envisions this as just the beginning of a new era in immune cell discoveries, suggesting that these maps will soon empower the design of therapies finely tuned to enhance T cell function for cancer treatments or suppress it for autoimmune disease management. The future of immunotherapy looks promising as these maps pave the way for more precise and effective interventions against a spectrum of challenging diseases.

## Unraveling the Flavonoid Metabolism: Scientists decode pathways for health in Plant-Powered diets

In a breakthrough revelation, an international research team has demystified the intricate process by which major flavonoids—naringenin, apigenin and genistein are metabolized within the human body. This critical insight not only clarifies the link between flavonoid metabolism and potential health benefits but also sheds light on the power of plant-based diets. As the world embraces plant-centric lifestyles, the significance of flavonoid-rich foods like broccoli, celery, and tofu is increasingly evident. Flavonoids, essential phenolic compounds in plant development, have long been believed to hold therapeutic and preventive potential against cancer and heart disease. Led by researchers Tsutomu Shimada and Professor Shigeo Takenaka, the team's molecular docking analyses reveal that human enzymes modify flavonoids in a manner akin to plant processes. Professor Takenaka emphasizes that these findings are foundational, unraveling the correlation between flavonoid metabolism and the health-boosting effects they may confer.



## Game Changer in Meet & Milk Adulteration Detection: ICAR-CIRC, Meerut Scores Patent for Rapid Test.

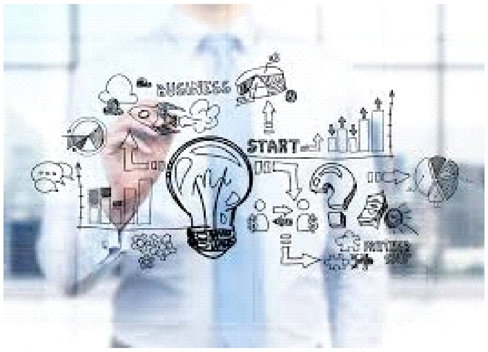
In a significant stride against adulteration, ICAR-Central Institute for Research on Cattle, Meerut has secured a patent (Patent No: 474562) for their groundbreaking invention titled "Development of an in-house built lamp assay for rapid detection of cow components adulterated in buffalo milk/meat" under the Patents Act, 1970. With concerns rising over the illegal mixing of cow meat with buffalo meat due to cow slaughter bans in various regions, this invention employs the loop-mediated isothermal amplification (LAMP) method for swift detection.

The innovative methodology ensures the rapid identification of cow components in buffalo milk or meat, offering a reliable solution to combat adulteration. Capable of detecting a minimum of 5% adulteration, the LAMP test stands out for its speed, cost-effectiveness, user-friendly nature, and precision in identifying adulterants. Its simplicity and specificity make it adaptable to any laboratory, promising a versatile tool for quick species identification in animal products. ICAR-CIRC's patent signifies a major breakthrough in the ongoing battle against food adulteration concerns.

## Empowering Entrepreneurs: ICAR-CIPHET's breakthrough Gluten-Free Tech Sparks Local Business Growth

In a stride towards meeting the nation's food needs, ICAR- Central Institute of Post-Harvest Engineering & Technology

(CIPHET) in Ludhiana has been unwavering in its commitment to developing and promoting healthy food products. Recently, their dedicated research team, led by Dr. Manju Bala, Dr. Arun Kumar T.V., and Ms. Surya Tushir, achieved a milestone in the creation of technologies for gluten-free maize-based food products. These innovations were showcased successfully across multiple platforms.



The impact of ICAR-CIPHET's breakthrough reached local aspiring entrepreneurs like M. Simranjit Kaur from Batala, who recognized the potential of this technology. Seeking to bring it to fruition, she approached ICAR-CIPHET. In recognition of her initiative, Dr. Nachiket Kotwaliwale, Director of ICAR-CIPHET Ludhiana, awarded a certificate to Ms. Simranjit Kaur upon the successful completion of training. Dr. Manju Bala, the principal inventor of this transformative technology, personally conducted hands-on training for the entrepreneur from November 28th to 29th, 2023, further emphasizing ICAR-CIPHET's commitment to fostering local businesses in the food processing sector.

### ICAR's Rotary Disc Drill for Crop Residue Management

A patent has been awarded for the innovative Rotary Disc Drill developed by ICAR-Indian Institute of Wheat and Barley Research in Karnal. This state-of-the-art machine introduces a novel approach to in-situ crop residue management, heralding a new era in conservation agriculture.

The Rotary Disc Drill is a versatile technology designed for direct seeding of various crops, including wheat, rice, soybean, pea, barley, green gram, and pigeonpea. What sets it apart is its ability to operate efficiently in the presence of anchored and loose crop residue such as rice straw, sugarcane trash, maize, and leguminous crop residue, ensuring minimal soil disturbance and reduced power requirements. Positioned to be a game-changer in regions like Punjab, Haryana, Uttar Pradesh, and beyond, where rice-wheat and sugarcane-wheat cultivation is prevalent, this innovation promises to address the critical issue of residue burning. With the patent secured, the Rotary Disc Drill is poised for commercialization through a licensing process, marking a significant leap forward in sustainable and efficient farming practices.

### Hidden Marvel of the Bay: ICAR-NBFGR Unveils a New Spotted Snake Eel Species

In a fascinating revelation, the ICAR-National Bureau of Fish Genetic Resources (NBFGR) has officially introduced the world to 'Ophichthus naevius' a new-found species of snake eel discovered along the picturesque coasts of Cuddalore in Tamil Nadu. The name, derived from the Latin term meaning 'spotted' or 'marked with moles,' perfectly encapsulates the distinguishing feature of dark spots embellishing its dorsal surface. The holotype of this captivating species now finds its home at the esteemed National Fish Museum and Repository of ICAR-NBFGR in Lucknow, forever catalogued for scientific inquiry.

This exciting discovery, marking the fourteenth addition to the Ophichthus genus in Indian waters, has been formally documented in the "Journal of Fish Biology," a reputable peer-reviewed scientific journal. The unveiling of 'Ophichthus naevius' not only enhances the richness of marine biodiversity but also underscores the critical role played by institutions like ICAR-NBFGR in unraveling the mysteries of India's Bay of Bengal.

## Shining a Light on Malaria: FAU Researchers Introduce Innovative Autofluorescent Compound in the Fight Against Drug Resistance

In the relentless battle against malaria, researchers at Friedrich-Alexander-Universität Erlangen-Nürnberg (FAU), under the guidance of Prof. Dr. Svetlana B. Tsogoeva, have pioneered a groundbreaking approach. Combining the potent anti-malaria drug artemisinin with coumarin, a plant-derived compound, the team has engineered an auto-fluorescent compound. This novel creation not only provides a real-time visualization of the medication's action within live cells but also boasts the remarkable ability to combat drug-resistant malaria pathogens, specifically plasmodium falciparum.

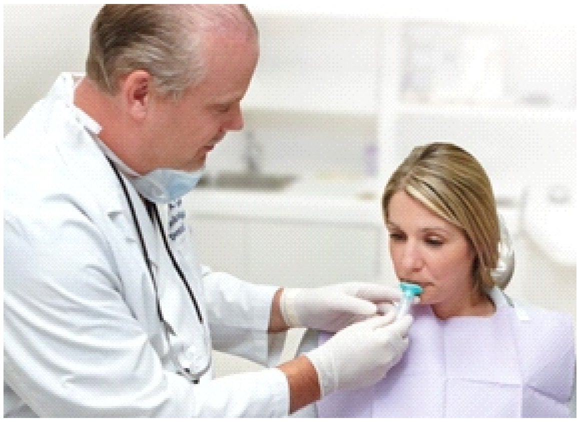
Artemisinin, extracted from the sweet wormwood plant, is a cornerstone in malaria medication due to its high efficacy. Coumarin, a secondary plant compound found in various plants, joins forces with artemisinin in the development of this innovative compound.

Published in the journal Chemical Science, the FAU team's findings not only illuminate the intricate workings of these autofluorescent artemisinin-coumarin hybrids but also offer a promising avenue in the pursuit of new compounds to combat the ever-present threat of drug resistance in malaria.

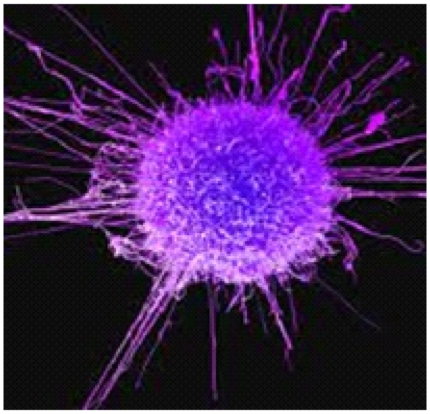
## Saliva Test for Early Cancer Detection

In a significant leap towards early cancer detection, researchers at the University of Gothenburg have unveiled a groundbreaking method that may make saliva a potent tool for identifying incipient cancer. The key lies in interpreting changes in sugar molecules, specifically glycans, which are linked to proteins in our cells. The structure of these glycans determines protein function, and alterations in their structure can signal inflammation or disease. The research team, led by Daniel Bojar, has developed a method enhanced by artificial intelligence (AI) to distinguish various types of structural changes in glycans, offering precise insights into specific diseases.

Analyzing data from 220 patients with 11 different cancers, the AI-enhanced method identified distinct glycan substructures associated with each cancer type. Unlike traditional statistical tests, the team's approach overcomes the limitations of sensitivity and reliability, as it considers the structural relationships between different sugars.



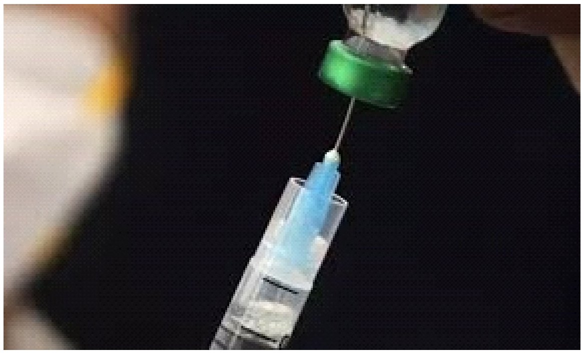
Bojar emphasizes the reliability of their statistically significant results and plans to use these newly discovered biomarkers to develop test methods. With a recent grant of SEK 4 million from the Lundberg Foundation, the team aims to acquire a state-of-the-art mass spectrometer, serving as an AI platform for studying glycans, with the ultimate goal of detecting cancer earlier, potentially through blood samples or saliva. Bojar envisions clinical tests on human samples within the next 4-5 years, offering a rapid and reliable analytical method for cancer diagnosis.



## Trivalent vaccine candidate fights measles, mumps, SARS-CoV-2

A potential game-changer in the fight against COVID-19 emerges as researchers at the University of Gothenburg propose an innovative trivalent vaccine. Leveraging altered measles and mumps viruses, the team explores the creation of a vaccine platform capable of triggering immunity against various SARS-CoV-2 variants. By inserting a stable segment of the coronavirus spike protein into these well-established viruses, the scientists aim to provide broad and enduring protection against COVID-19.

In a study published in Proceedings of the National Academy of Sciences, the researchers describe the MMS vaccine candidate (Measles, Mumps, and SARS-CoV-2) as a nasal delivery system that induces robust neutralizing antibody responses. Tested in rodent models, the intranasal vaccine not only prevents disease symptoms but also demonstrates prolonged immunity, lasting at least four months without decline. The trivalent vaccine exhibits promising efficacy against ancestral SARS-CoV-2, delta, and omicron variants, emphasizing its potential to combat evolving strains.



“The beauty here is we already know the MMR is used in children, so we’re building on a 50-year safety record,” notes Jianrong Li, the senior author of the study. The MMS approach presents a novel strategy, capitalizing on the safety of the measles-mumps-rubella (MMR) vaccine while introducing three distinct spikes that generate broad neutralizing antibodies. With further research and development, this trivalent vaccine could emerge as a reliable and innovative tool in the ongoing battle against COVID-19.

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Sirova, D., Adamec, L. and Verba,J. (2013). Enzymatic activities in trops 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 need for future. Procedings 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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