EXPLORE

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DEPARTMENT OF BOTANY

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EDITORIAL

It is indeed a proud moment for me to introduce 5th Edition of the Departmental Journal 'EXPLORE' published biannually by the Department of Botany, Shri Shikshayatan College.

The Department of Botany of this College has been instrumental in inculcating original and innovative thinking among the students since its establishment in 1956 and has walked through a long way to establish a niche of its students spread far and wide in corners of this nation as well as across the borders.

Since its inception, 'EXPLORE' has tried to provide the current students of the department a platform to showcase their scientific aptitude and critical thinking and has trained them to adapt to their future endeavors in the scientific world. It has also been a medium of exchange between the past and the present students as the alumni of our department have significantly contributed by sharing articles about their pursuits after graduating from the College.

This year has not been an exception. The articles contributed by the current as well the past students of the Department have summed a huge array of scientific research going around in the field of biological sciences.

On behalf of our department, I would like to extend my sincere thanks to the Management of our College and our Principal, Dr. Tania Chakravertty for their constant encouragement and support in making this publication possible. We pledge to continue this effort in future and strive for its betterment.

DR. SUCHHANDA GHOSH
Head, Department of Botany
Shri Shikshayatan College

CONTENTS

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DEPARTMENTAL JOURNAL EXPLORE

Adeeba Dahlech

Batch of 2009-2012, Post Doctoral Researcher, Stanford University

Hi everyone! My name is Adeeba and I graduated from Shri Shikshayatan College in 2012 with an Honours in Botany. I want to begin by thanking you for reading the experiences that I've gained in the past 12 years. Sometimes it just feels like yesterday that my friends and I were crunching hours in the library making notes on angiosperms. Yet, somehow, I am typing my past and current experiences sitting in an office on campus at Stanford University. I've worked on varied projects during my PhD and post-doctoral work, but my primary focus has always been RNA viruses. RNA viruses are viruses that have RNA instead of DNA as their main genetic material and are some of the most common viruses infecting humans worldwide. They include significant pathogens like poliovirus, rhinovirus, enteroviruses (EV) A-71 and D-68, Dengue, Zika, and corona virus. Although some, like poliovirus and SARS-CoV-2, can be controlled by vaccination, vaccines are not available for others and few effective antivirals exist. This makes RNA viruses one of the crucial areas of study not only to combat current infections and outbreaks but also to mitigate any potential risks and develop pandemic preparedness.

For my PhD work at Indiana University, School of Medicine, I studied enteric viruses. These are highly infectious human pathogens that initiate infection in the gastrointestinal tract. They are transmitted through the fecal-oral route and are primarily spread by contamination of food and water sources. Due to the ease of transmission, these viruses are a significant cause of sickness and death in humans, especially infants and children. Further, enteric viruses also cause a substantial economic burden each year. Of the enteric viruses, I focused on Coxsackievirus which is commonly isolated among enteric viruses and causes myocarditis (inflammation of the heart) and HFM (hand, foot, and mouth) disease. Despite Coxsackievirus' impact on human health, there is a lack of treatment options or vaccines to prevent Coxsackievirus infections.

Using a mouse model to study a key Coxsackievirus, Coxsackievirus B3 (CVB3), I was able to show three critical factors that impact CVB3 replication and pathogenesis. First, along with fellow lab members, I demonstrated that intestinal bacteria (bacteria found in our gut) can enhance intestinal CVB3 replication. We found that certain specific bacteria and their bacterial cell wall components enhanced CVB3 stability and infectivity. These data suggest that specific bacteria may be integral in maintaining CVB3 infectivity in the intestine (1). In my second and novel finding, we determined that sex influences intestinal replication of CVB3 and the subsequent immune response. Our data suggest that testosterone, a predominant male sex hormone, enhanced CVB3 intestinal replication and viral dissemination to organs in both male and female mice. Moreover, testosterone also affected the immune response by reducing the activation of the CD8 T cells, which kill infected cells, in response to CVB3 infection (2). In contrast, we found an enhanced CD8 T cell response in female mice to CVB3 infection, suggesting a sex-dependent T cell response that may underlie the sex bias in disease (3, 4). In addition to that, we also discovered a novel CD4 T cell immune response in female mice to CVB3 infection. We found that CVB3 induces an expansion of a subset of CD62L Lo CD4 T cells in the lymphatic system of female but not male mice. This response was specific to CVB3 viral antigen and depleting these CD4 T cells prior to infection made the female mice susceptible to death, indicating that CD4+ T cells play a protective role against CVB3 in our model (5). Overall, these data represent an important advancement in the Coxsackievirus field and will help in developing future therapeutics and aid in vaccine design to limit Coxsackievirus infections.

Currently, as a post-doctoral scholar at Stanford University School of Medicine, I continue to work with RNA viruses but have changed my focus to flaviviruses, especially Dengue and Zika. I did this for several reasons. First, to broaden my knowledge of RNA virology and also to address the elephant in the room- drug resistance. Most RNA viruses are prone to mutations as they lack a machinery that can detect errors in the genome and thus give rise to new viral strains with additional cycles of replication. As a result, different strains of the same virus may exist within the same host. An issue with having different strains is that some of them may develop drug-resistant mutations and not be inhibited by the available antivirals, making drug resistance a major problem (6). Thus, a better strategy to combat drug resistance and block the production of new viral variants is required.

Flaviviruses such as dengue and Zika viruses initiate their replicative cycle by translating the RNA genome into a single polyprotein which is then processed by host and viral proteases to produce individual viral structural and non-structural proteins and their respective precursors. Studies from our lab have shown that if we alter this polyprotein processing by targeting individual cleavage sites within the polyprotein, precursors can accumulate that will be inhibitory to all viruses within the same cell (7). Flavivirus protease NS2B/3 is responsible for many cleavages in the dengue polyprotein; three of its cleavages are intra-molecular or cis-acting in nature which means that the protease self-cleaves the polyprotein. By specifically targeting a subset of these intra-molecular sites, we can accumulate toxic precursors that inhibit viral growth, making NS2B/3 a promising drug target (7).

Our laboratory has entered an exciting collaboration with the National Institute of Health Antiviral Drug Discovery Center and the AI-driven Structure-enabled Antiviral Platform (ASAP) to develop compounds to inhibit the NS2B/3 protease sites of Dengue and Zika. I am studying and testing these potential antivirals to identify the specificity of their cleavage inhibition in the viral polyprotein and also research which activity and function of the compounds correlate with viral inhibition. We have identified specific precursors whose accumulation correlates with potent antiviral activity. Using these tool compounds, my goal is to identify drug-resistant viruses and study the mechanism of how the precursors limit the growth of these drug-resistant viral strains as they emerge in infected cells and what is their overall relevance in the Dengue and Zika life cycle.

A deeper understanding of RNA biology could serve as a basis for more rational drug design for all viruses that utilize a polyprotein strategy. Additionally, there are several other RNA viruses like EV-D68 and EVA-71 that have precursors of viral protease like 2A and 3C which need to be tested for intramolecular cleavage events (8). Determining how small molecules differentially inhibit protease activity based on their interactions and exploring the mechanism behind protease inhibition based on structural interactions and kinetics will help prioritize efforts to evade the emerging problem of drug resistance in the development of next-generation antiviral strategies.

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WATER HYACINTH AND ITS USE IN PHYTOREMEDIATION

Anushka Mukherjee, Anwesha Chattopadhyay, Teesta Ghosh, Rupsa Dey, Santosi Singh

Semester V

Pure water is essential for the survival of all living beings. Known as the "elixir of life," it is vital for the physiological and metabolic processes of every organism, including humans. Despite its immense importance, the scarcity of potable water is slowly but surely becoming a global issue. Therefore, the conservation and restoration of this resource are of the utmost importance.

Conventional waste water treatment methods include trickling filters and activated sludge processing. They combine physical, chemical, and biological processes and operations to discard solid, liquid, and organic matter. The different methods of conventional waste water treatment include an inlet chamber, grit chamber, distribution chamber, anoxic tank, aeration tank, secondary clarifier, centrifuge, polyelectrolyte dosing system, and biological filters. Due to the widespread usage of such units at Sewage Treatment Plants (STPs), they require a large amount of electricity. The efficiency of these plants decreases drastically when faced with common problems such as power cuts and load shedding. The removal of humus is difficult through these processes.

A major drawback of traditional STPs is the significant capital, energy, and labor investment required. As a result, discovering more eco-friendly treatment methods is imperative. One possible answer to this issue may lie in one of the most common fresh water weeds : Water Hyacinth.

Phytore mediation may be defined as "using green plants and the associated microorganisms, along with proper soil amendments and agronomic techniques to either contain, remove or render toxic environmental contaminants harmless." The term comes from the Greek word *phyto*, meaning plant, and the Latin word *remedium*, meaning restoration of balance. Theoretically, it may be a plausible alternative to the commonly used cost-intensive processes.

The principle of a phytore mediation system is to clean up contaminated water. The most important factor in implementing it is selecting an appropriate plant that has a high uptake of organic and inorganic pollutants, grows well in polluted water, and can be easily controlled in quantitatively propagated dispersion. Now, this is where water hyacinths (*Eichhornia crassipes*) come in. One of the most common aquatic plants in India, they form vast green mats stretching over large areas on the surface of water bodies. The problems associated with this rapidly growing weed include the decline of biodiversity, blockage of rivers and drainage systems, depletion of dissolved oxygen, and environmental pollution. This ability to withstand various conditions and continue prolific growth makes them excellent candidates for this endeavor.

Eichhornia crassipes is a perennial aquatic macrophyte. A member of the family Ponderiaceae, it is a vascular plant with rounded, upright, waxy leaves and violet flowers. Today, it is one of the fastest-growing plants known, with the ability to double its size in as little as two weeks. The optimal water pH for the growth of this aquatic plant is neutral, but it can tolerate pH values from 4 to 10. This is vital, as it points to the possible usage of water hyacinth for waste water treatment.

Water hyacinth has demonstrated its ability to remove nutrients and other chemical elements from sewage and industrial effluents (Saltabas and Akcin, 1994). In their study, 35.00 ppm copper, 14.00 ppm nickel, and 10.00 ppm chromium solutions were used to observe the absorption capability of this plant. Due to the positive results obtained, they proposed the use of water hyacinth for the treatment of industrial effluents.

Haselowetal. (1992) indicated that controlled cultivation of this weed in lakes and ponds may improve the water quality. Jebanesan (1997) conducted an experiment where wastewater from the dairy industry was treated with water hyacinth for a retention period of 25 days, after which the calcium, magnesium, and total hardness were reduced by 47.5%, 54%, and 33% respectively, with a marked decrease in water chloride, chromium, nitrous and nitric nitrogen, pH, alkalinity, BOD, COD, bicarbonates, and specific conductance when compared to control.

In an investigation, a natural shallow eutrophicated wetland heavily irrigated by domestic sewage and agricultural runoff in Ujjain, a city in Madhya Pradesh, India, was studied (Billore et al., 1998). This area was also infested with water hyacinth. This study aimed to determine the role of the hyacinth in removing particulate matter attached to the root system, and nitrogen as contained in the root-attached particulate matter (RAPM) and in the plant tissue. The recorded hyacinth density was 79 plants per square meter with 1.549 kg dry plant tissue. They found that the plant in one square meter could retain 663 g RAPM (about 42% of dry plant tissue), which was potentially removed upon the mechanical harvest of the plants on a per-meter square basis. In addition to the significant amount of RAPM, the harvested hyacinth plant also brought about the removal of 1.396 g of organic matter, 0.536 g of total nitrogen, 0.482 g of ammoniacal nitrogen, and 0.338 g of nitrate nitrogen per sq. m of waterscape basis. The extensive root systems of the hyacinth provide a wide surface area for attached particulate matter and microorganisms, acting as a "suspended sediment layer" and rich in nitrogen. Thus, for a eutrophicated wetland receiving wastewater rich in particulate matter, the hyacinth growth has substantial potential for the removal of particulate matter and nitrogen through their attachment to roots, in addition to the nitrogen concentrated in the plant tissue.

Ayade (1998) reported that research on municipal sewage management in developing countries has utilized water hyacinths to purify sewage for potential domestic reuse. However, the ability of these plants to remove pollutants from raw sewage effluents has been hindered by sewage toxicity. To address this issue, a bio-degrader composed of various bacterial species, including Pseudomonas aeruginosa, Escherichia coli, Klebsiella ozaenae, Klebsiella edwardsii, and Bacillus subtilis, was introduced to adapt the plants. The adaptation process involved gradually increasing the sewage concentration from 20% to 40%, then to 60%, and finally to 80%, resulting in plants that could thrive in 100% sewage concentration. Over a four-week period, the plants were monitored for morphological growth, and tissue samples were collected for analysis at the end of this period. Initially, the plants effectively absorbed nutrients from the sewage; however, by the fourth week, signs of toxicity began to appear, evidenced by wilting, loss of turgidity, and a reduction in leaf count. Despite these challenges, the plants that survived the adaptation process across various sewage dilutions exhibited robust growth in raw sewage. When working in synergy with the active bio-degrader, the adapted water hyacinths demonstrated a remarkable 93% efficiency in removing pollutants (nutrients) from raw sewage.

In addition to wastewater treatment, water hyacinth serves as a viable alternative fuel source, can be converted into biomass briquettes for power plants, and is useful as compost, fertilizer, and animal feed for livestock. Identifying various applications for these aquatic plants not only aids in controlling their population but also mitigates their environmental impact. Ultimately, water hyacinths are fascinating plants that offer potential solutions to many of today's pressing challenges.

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HEAR OUT ! PLANTS ARE CALLING !

Dr. Asmita Pal

Batch of 2010-2013

Remember when Acharya Jagadish Chandra Bose in 1901 showed that just like any other life forms, plants can feel pain!

The present-day researchers reinforce his work and prove that not only we humans and animals can cry, but plants can too! Although humans might not be able to perceive this acoustic sounds, some animals can and they respond as well. Latest study by Khait et al., 2023 show sounds emitted by plants when thirsty or under some forms of stress are airborne and informative. When in stress, plants have been known from ages to display different kinds of phenotypic (or observable) changes, which produce visual, chemical, and tactile cues, however the ability of plants to produce sounds is new to science and an absolutely fascinating one!

Even if the sounds emitted by plants are not directly produced, but is a result of the plant's physiological condition, nearby organisms capable of hearing these sounds could use them for their own benefit. Research has shown that plants emitted airborne sounds lie in the ultrasonic range of 20–100 kHz and are able to be detected from several meters (3-5 meters) away, both in acoustic chambers and in greenhouses. The sound emissions by plant are perceived by many mammals and insects (e.g., mice and moths). Most interesting fact is that these emitted sounds carry information about the physiological state of the plant. Using machine learning models, scientists were able to distinguish between drought-stressed, cut, and control plants, based only on the sounds they emit. The cellular mechanism that scientists think may be responsible for the sound emission at least part of the sounds is probably cavitation in the stem. These sound emissions are potent enough to be applied for monitoring plants in field or greenhouse. More specifically, plant sound emissions can be utilized to monitor watering of crops and also possibly to understand disease states – significantly crucial topics in agriculture. The ability to distinguish between drought-stressed and control plants based on plant airborne sounds open up a novel avenue of research in the field of precision agriculture.

Lastly, not only can plants speak, they can hear too. Plants have been demonstrated to respond to sounds, by changing the expression of specific genes, or by increasing sugar concentration in their nectar. Such interesting phenomenon is what makes plants probably one of the most intelligent life forms on Earth and humans still are only beginning to understand them!

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Khait, I., Lewin-Epstein, O., Sharon, R., Saban, K., Goldstein, R., Anikster, Y., ... & Hadany, L. (2023). Sounds emitted by plants under stress are airborne and informative. ***Cell,*** 186(7), 1328-1336.

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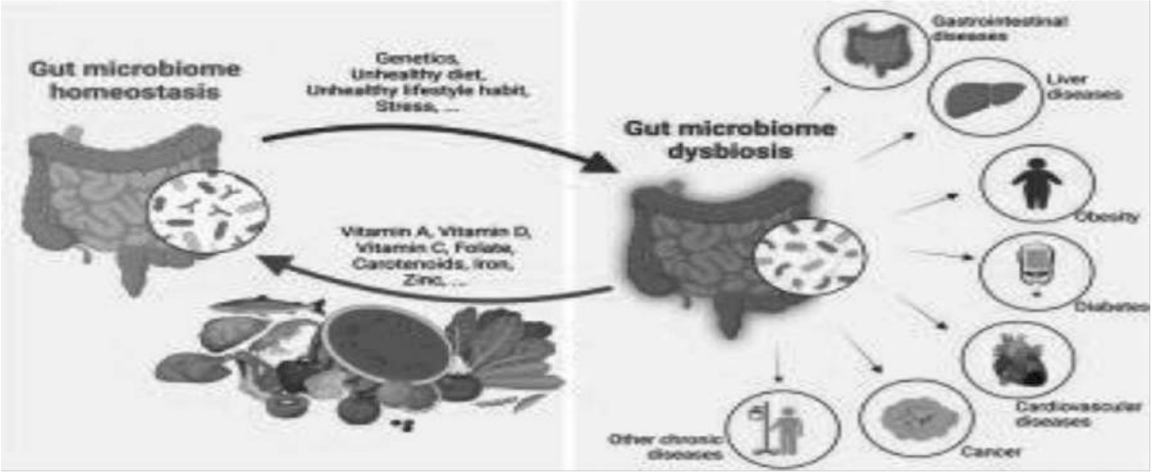
MICROBIOME RESEARCH AND HUMAN HEALTH

Atrija Sarkar, Aanchal Mundhra, Gungun Jain, Stuti Beria
Semester V

Introduction - The symbiotic and pathogenic microbial community which resides inside our body and our skin (the human microbiome) can perturb host energy metabolism and immunity, and thus significantly influence development of variety of human diseases. Therefore the field has attracted unprecedented attention in the last decade. The human microbiota is defined as the genomic content of organisms inhabiting a particular site in the human body. From childbirth, a steady symbiosis between human body and its indigenous microbiota begins. These interactions play an important role in maintaining general health and wellbeing. The human microbiome is constantly evolving in response to host factors such as age, nutrition, lifestyle, hormonal changes etc. A balanced microbiota has shown to play an important role in human sustenance. In general, the works aims to review and discuss the impact of the human microbiome on human disease and on maintaining health.

What is Human Microbiome ? The human microbiome is the genetic material of all microbes, e.g. Bacteria fungi protozoa and viruses which live on inside the digestive tracts of humans and other animals. It has evolved with its host for millennia and therefore has been extensively involved with a variety of essential activities in the host, e.g. digestion, detoxification, disease mediation etc. Consequently, a large number of microbes with high diversity can be found in mammalian gut, with most of them being *Firmicutes* and *Bacteroidetes.*

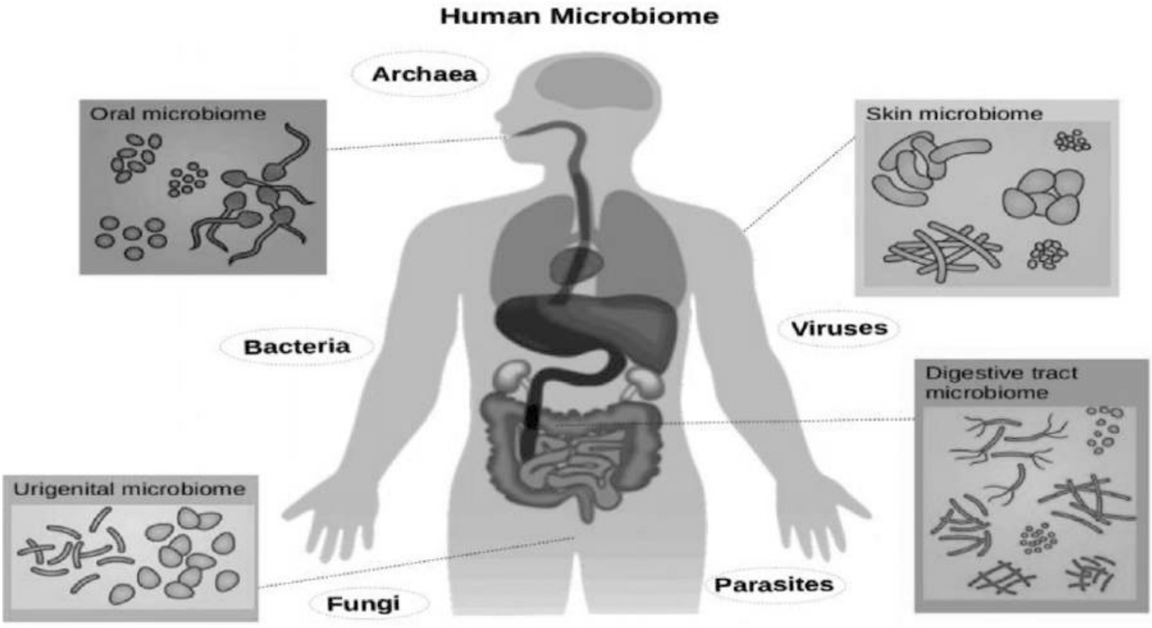
With diverse microorganisms, the human microbiome contains millions of different genes. Some of them may be acquired from environmental bacteria, indicating their metabolic diversity and versatility. In general, host diet and phylogeny contribute to modifying the composition of gut microbial communities in mammals and other species. Indeed, genome scale metabolic modelling shows the variations in the diet of the host significantly modified the composition of three representative human gut bacteria (*B. thetaiotaomicron, E. rectale and M. Smithii*).

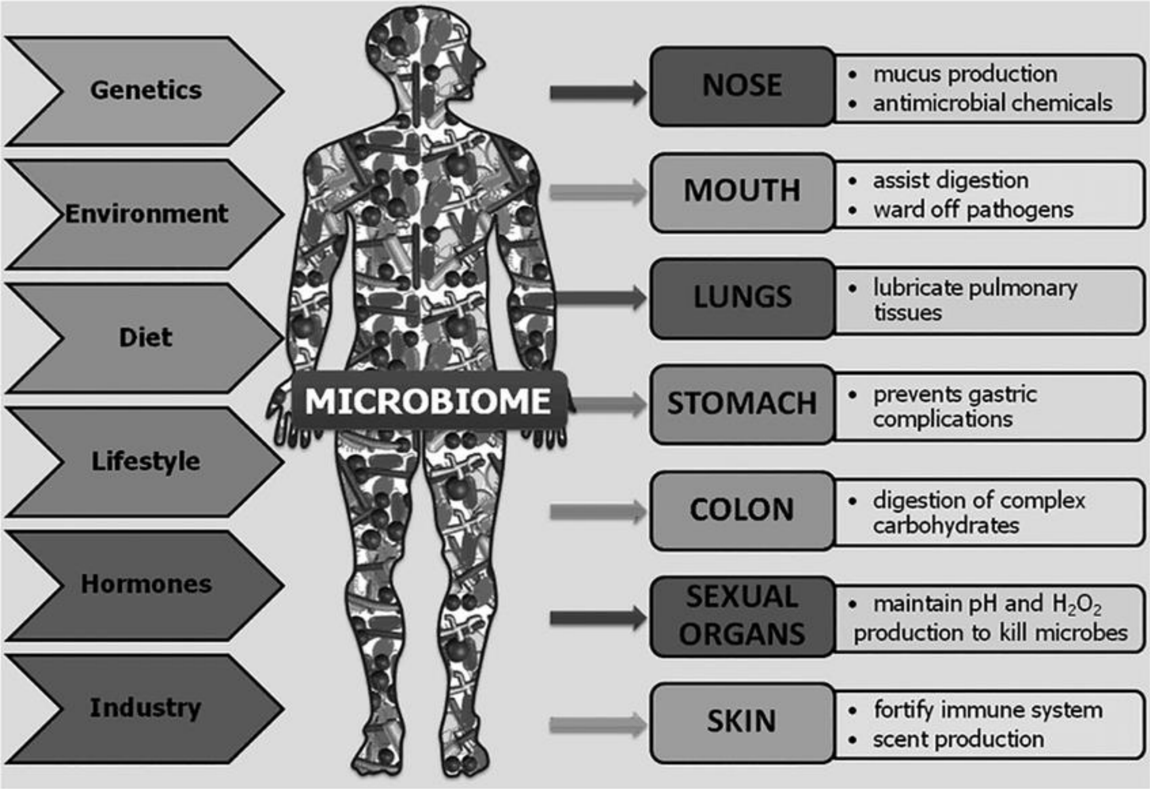


Factors Affecting Human Microbiome :

- **Diet :** A diet rich in fibre, fruits, vegetables and fermented foods promotes a diverse and healthy microbiome. High fats and processed foods may lead to dysbiosis (microbial imbalance) and health issues.
- **Antibiotics and medications :** Antibiotics can disrupt the balance of microbiome by killing both harmful and beneficial bacteria.
- **Mode of Birth (Vaginal vs. caesarean Section) :** Babies born vaginally are exposed to their mother's vaginal and gut microbes, which influences the early development of microbiome. Caesarean section birth may result in less initial exposure to maternal microbes, potentially affecting the infant's microbiome diversity.
- **Infection and disease states :** Certain infections or chronic conditions like inflammatory bowel disease can alter microbiota composition. Disease states can shift the microbiome towards dysbiosis.
- **Lifestyle Factors :** Exercise, sleep, and stress levels also play roles. Regular exercise is associated with higher microbial diversity, while stress and poor sleep can disrupt gut microbiota, potentially impacting mental and physical health.
- **Genetics :** Genetics influence microbial composition, but this effect is generally weaker compared to diet and lifestyle. Certain genetic traits may predispose people to specific microbial compositions, which may impact health risks.

Understanding these factors helps guide research on potential ways to modulate the microbiome to improve health factors.





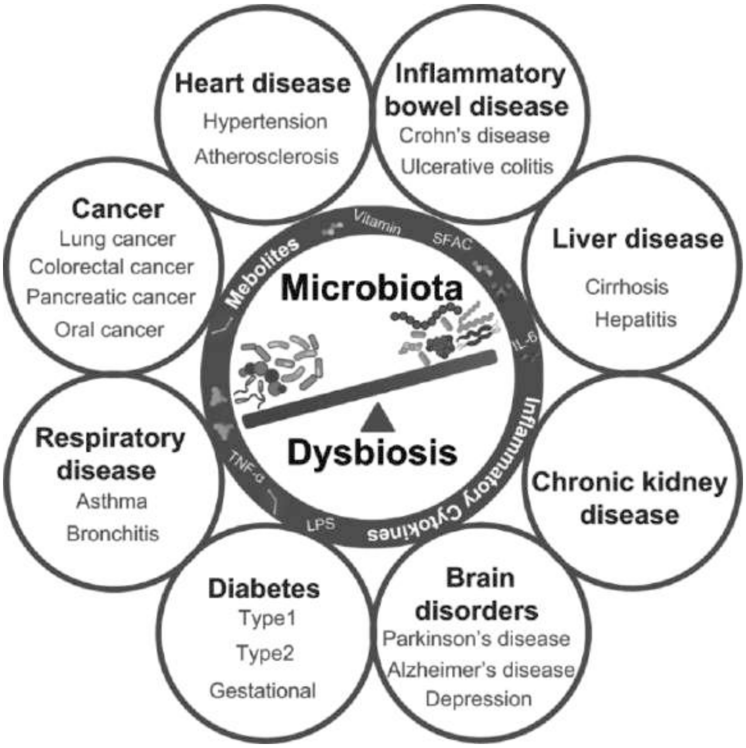
Key roles of microbiome in human health :

1. **Digestive health –** The gut microbiome helps breakdown complex carbohydrates, fibres, and other substances that human enzymes cannot process, aiding in digestion and absorption of nutrients. The stomach was believed to be a sterile organ due to its inhospitality of bacteria. The gastric juice has a diverse microbial community that differs from gastric mucosa. The dominating phyla in gastric juice are Firmicutes, Actinobacteria, Bacteroidetes, whereas Proteobacteria and Firmicutes are dominant in gastric mucosa. Some of the commonly studied microbiome associated diseases are ulcerative colitis, Crohn's disease, Irritable bowel syndrome, celiac disease, colorectal cancer etc.
2. **Mental health (Gut-brain axis) –** There is increasing evidence that microbiome impacts mental health through the gut-brain axis. The microbes in the gut can produce neurotransmitters like serotonin, which is involved in mood regulation. An imbalance in gut bacteria has been linked to conditions like depression, anxiety, and even neuro developmental disorders like autism. The GBA plays an important role in maintaining homeostasis and modulating the function of central and enteric nervous systems.
3. **Skin health –** The skin microbiome is a collection of microorganisms that live on skin and play a vital role in maintaining skin health. Its help in maintaining the skin's barrier function, which protects the skin from pathogens and toxic substances. It educates the immune system to

tolerate beneficial microbes while fighting pathogens. An imbalance in the skin microbiome leads to skin diseases such acne, eczema, psoriasis and wound functions.

4. **Urinary health –** The urinary tract microbiome, or urobiome is a vital part of urinary health. It helps maintaining bladder homeostasis, prevents urinary tract infections and promote local immune function. Change to urobiome have been linked to diseases like as neurogenic bladder dysfunction, interstitial cystitis and urgency urinary incontinence.

This is how the large and diverse group of microorganisms that reside in various parts of human body have a highly coevolved relationship with human health. Microbiome research has highlighted the importance of human microbiota ecosystems in the promotion of health and various disease causing processes.



Conclusion :

The study of human microbiome is important and it gives as in depth understanding of the interplay between humans and its indigenous microbiota. This gives valuable insight into further research studies in optimizing these organisms to combating life threatening diseases. It is important to note the continuous use of broad-spectrum antibiotics may disrupt the human microbiota. This results in an imbalance of the indigenous microbial community paving way for invading pathogens. However, treatments with the use of pre and probiotics should be encouraged. Hence more research should be focused on the use of probiotic therapy in treatment of infectious disease. In addition, further studies should emphasize on the effect of the human microbiome on mental health and also the impacts of mycobiome and virome community on indigenous microbiota as they may contribute to dysbiosis.

VAM, ITS ROLE IN NUTRIENT UPTAKE OF VASCULAR PLANTS AND ISOLATION PROCEDURES

Disha Ram, Priyanka Kumari, Sudeeksha Jaiswal, Ishika Ghosh Dastidar
Semester I

Abstract

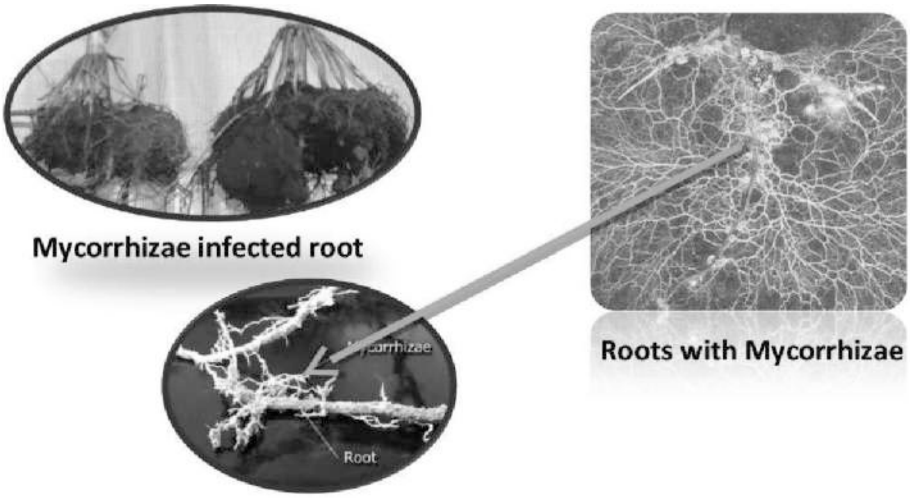
Symbiotic fungi are quite helpful for the plants to fixate on certain nutrients from the surrounding. One such example is Vesicular Arbuscular Mycorrhiza. It is commonly recognized as VAM. It is present in the roots of almost all vascular plants and play a decisive role in the mineral nutrition of plants. Techniques for obtaining VAM samples from natural sources are outlined and methods of identifying the fungal structures are discussed.

Introduction

Fungi which develop beneficial symbiotic associations with the roots of vascular plants and contribute majorly to plant growth are called Mycorrhizal fungi. In this interaction, both organisms benefit from one another in a way that the plant supplies the fungi with photosynthetic products and fungus supplies the plant with nutrients.

Vesicular Arbuscular Mycorrhizae (VAM) association is the most prevalent and important of these mutualistic associations. It forms external hyphal network in the soil, growing extensively within the cells of the root cortex and are so-called because of the two characteristics structures: vesicles and arbuscules- found in roots with this type of infection. VAM have been placed under the Order **Glomales**.

VAM fungi are mainly used in crop plants and improves the absorption of uptake of water, minerals and particularly the poorly mobile ion phosphorus from the soil and appreciably increase the growth of plants.



Structure of VAM :

The structure of VAM involves both the fungal and plant components :

Fungal Components :

Hyphae : These are filamentous structures that form the main body of the fungus. They penetrate the soil and extend from the root surface, increasing the plant's absorptive surface area.

Vesicles : These are spherical structures formed by the fungus within the root cells. They store lipids and other nutrients, which can be transferred to the plant.

Arbuscules : These are tree-like structures formed by the fungus within the root cells. They increase the surface area for nutrient exchange between the fungus and the plant.

Spores : These are reproductive structures of the fungus that are released into the soil, where they can germinate and infect new plant roots.

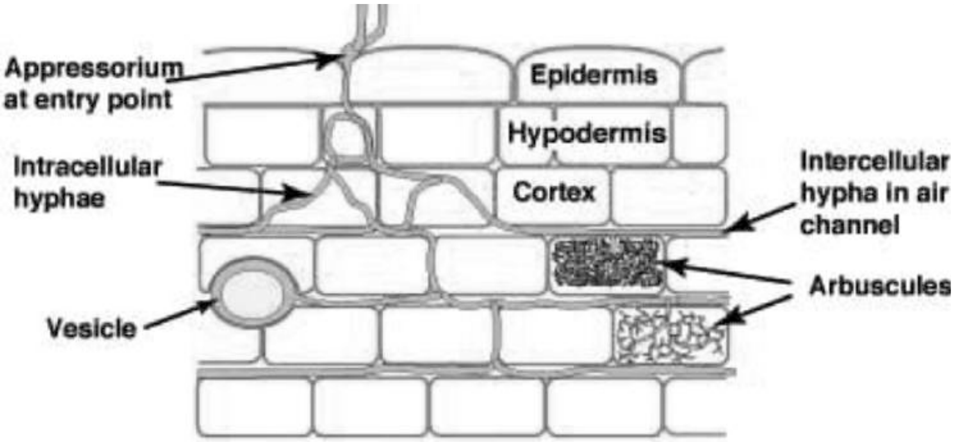


Fig: Structural Components of Vesicular-Arbuscular Mycorrhizae (VAM)

Plant Components:

Root : The root provides a physical support for the fungus and supplies it with carbohydrates produced by photosynthesis.

Root Cells : The root cells are penetrated by the fungal hyphae, which form vesicles and arbuscules within the cells.

Interactions of VAM with various plants

Vesicular-Arbuscular Mycorrhizae (VAM) form symbiotic associations with a wide variety of plants. Here are some examples of plant groups that commonly associate with VAM:

1. **Grasses :**

   o Example : Wheat (*Triticum spp.*) and corn (*Zea mays*) benefit significantly from VAM for nutrient uptake.

2. **Legumes :**

   o Example : Clover (*Trifolium spp.*) and soybean (*Glycine max*) can enhance nutrient acquisition through VAM associations.

3. **Fruit Trees :**
   o Example : Apple (Malus domestica) and grape (*Vitis vinifera*) show improved growth and fruit production with VAM.
4. **Vegetables:**
   o Example : Tomato (*Solanum lycopersicum*) and pepper (*Capsicum spp.*) have enhanced growth and resistance to certain stresses when associated with VAM.
5. **Ornamental Plants :**
   o Example : Many flowering plants, like marigold (*Tagetes spp.*) and petunia (*Petunia spp.*), benefit from VAM associations.
6. **Herbs :**
   o Example : Basil (*Ocimum basilicum*) and mint (*Mentha spp.*) can have improved growth and flavor when associated with VAM.
7. **Perennials :**
   o Example : Echinacea (*Echinacea purpurea*) and other native perennials often form beneficial relationships with VAM.

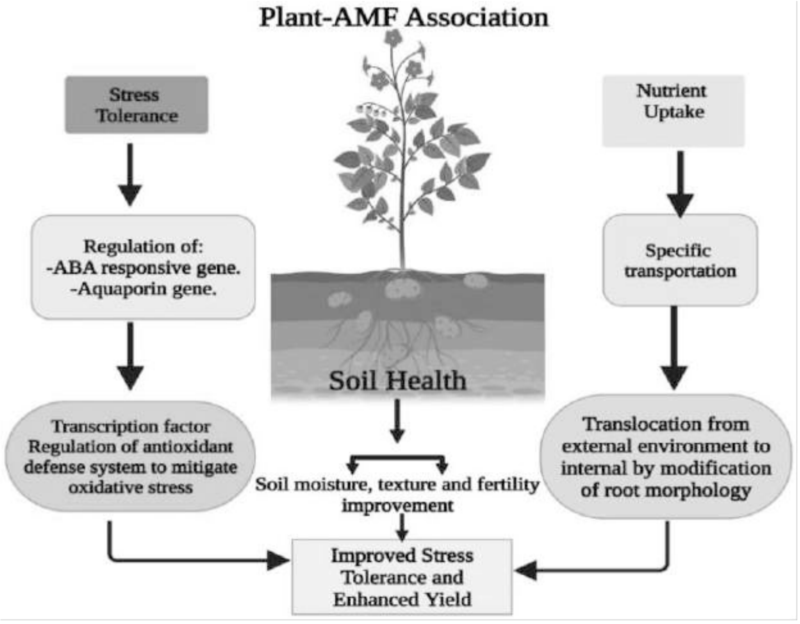
These associations are vital for nutrient acquisition and overall plant health, especially in nutrient-poor soils.

Highly magnified view of a *Glomus* Arbuscule.

Different functions of VAM

Primarily the symbionts trade nutrients, and the arbuscular mycorrhizal (AM) fungus obtains carbon from the plant while providing the plant with an additional supply of phosphorus (as phosphate). While much research has focused on nutrient exchange, the VAM symbiosis is associated with a range of additional benefits for the plant including the acquisition of other mineral nutrients, such as nitrogen, phosphorus and resistance to a variety of stresses such as drought, soil/root borne pathogens, salts, heavy metals and soil stability.

The concept of using VAM fungi as a bio fertilizer, in terms of cost effectiveness, energy saving and as environment friendly, is a promising perspective and its significance in augmenting food production is far and wide, therefore it can be used in sustainable agriculture.



Nutrient uptake

Microbial activity in the rhizosphere is a major factor that determines the availability of nutrients to plants and has a significant influence on plant health and productivity. The potential of symbiosis has contributed much to the nitrogen and phosphate nutrients in the natural ecosystems, particularly on infertile sites or in recently denuded areas. The AM fungi have been found to release an unidentified diffusional factor, known as the *myc factor*, responsible in activating the nodulation factor's inducible gene MtEnod11. This is the gene which is involved in establishing symbiotic relationship with the **Nitrogen** fixing, rhizobial bacteria. The enhanced nitrogen status of the plant promotes further development of the mycorrhizal symbiosis.

VAM are obligate symbionts. They have limited saprobic ability and are dependent on the plant for their carbon nutrition. VAM fungi take up the products of the plant host's photosynthesis as carbon sources (i.e hexoses, fructose and sucrose) and efficiently utilize or store it. AM fungi are important to their hosts as they enhance the ability of plants to absorb **Phosphorus** from soil, which is relatively inaccessible to the plants. An increase in the carbon supplied by the plant to the VAM fungi increases the uptake of phosphorus and the transfer of Phosphorus from fungi to plant. Evidences indicate that VAM colonized plants absorb and accumulate more phosphorus compared to non-colonized plants when plants are grown in soils that are low in phosphorus . This increase in uptake may be due to increase surface area of soil contact, increased movement of nutrients into mycorrhizae, a modification of the root environment and increased storage. Not only the uptake of Phosphorus is enhanced by VAM colonization of plant roots, the uptake of other macro and micronutrients like Ca, Mg, S, Cu, Fe, Zn and B have also been enhanced.

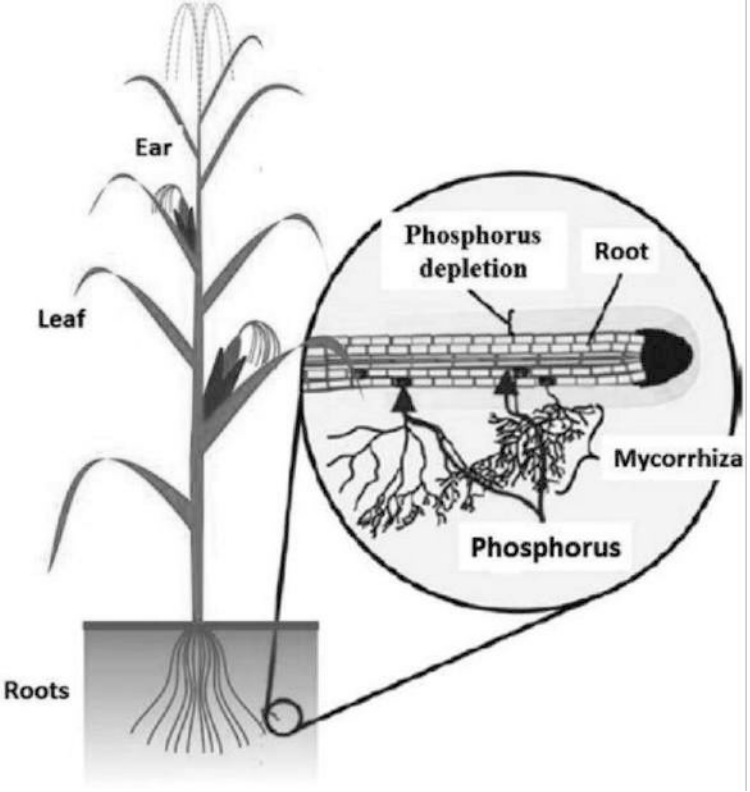


Fig: Association of VAM in Phosphorus Solubilisation

The manipulation of crop rhizosphere with PGPR for bio-control of plant pathogens has been found promising. VAM associations have been of significant help to crop production and soil fertility as reported by many researchers across different agro-ecological zones in many countries. The biofertilizer properties of plant growth promoting rhizobacteria (PGPR) are frequently ascribed to their ability to increase the bioavailability of inorganic and organic phosphorus and some of these bacteria have documented synergistic effects on nitrogen fixation and formation of mycorrhizal associations which can further proved by Toro et al. (1997) demonstration.

He observed that both *Enterobacter sp.* and *Bacillus subtilis* promoted establishment of the AM fungus *Glomus intradices* and increased plant biomass as well as tissue nitrogen and phosphorus contents. Phosphorus content in tomato plants was increased when inoculated with the AM fungus *G. etunicatum*, or the phosphate solubilizing bacterium Enterobacter agglomerants (Kim et al., 1997). Additionally, the highest nitrogen and phosphorus uptake was observed when tomatoes were inoculated with both the organisms, suggesting that bacteria and AM fungi might together increase the rate of nutrient uptake by the plants. Root colonization by VAM fungi is also a unique area that has justified the potential of VAM as bioprotectant and as biofertilizer providing protection to plants from parasitic fungi and nematodes and also increase plant growth and yield.

Bio-control agent

Through various demonstrations and studies conducted, VAM has also shown a reduction in pathogen population or in the severity of disease in the host plant against nematodes and fungi. Upon root colonization by VAM fungi, profound physiological changes occur in the host plant. Progressive binding of phenolic compounds in VAM infected roots is directly involved in the control of VAM endophytic establishment and development as it gradually reduces the plasticity and elasticity of the symbiotic matrix. Phenolic compounds bound to cell walls could also be indirectly responsible for the resistance of VAM roots to pathogenic fungi, since they result in increased resistance by cell wall to the action of digestive enzymes. Mycorrhizae even promote beneficial bacteria that may be directly responsible for protection against root pathogens.

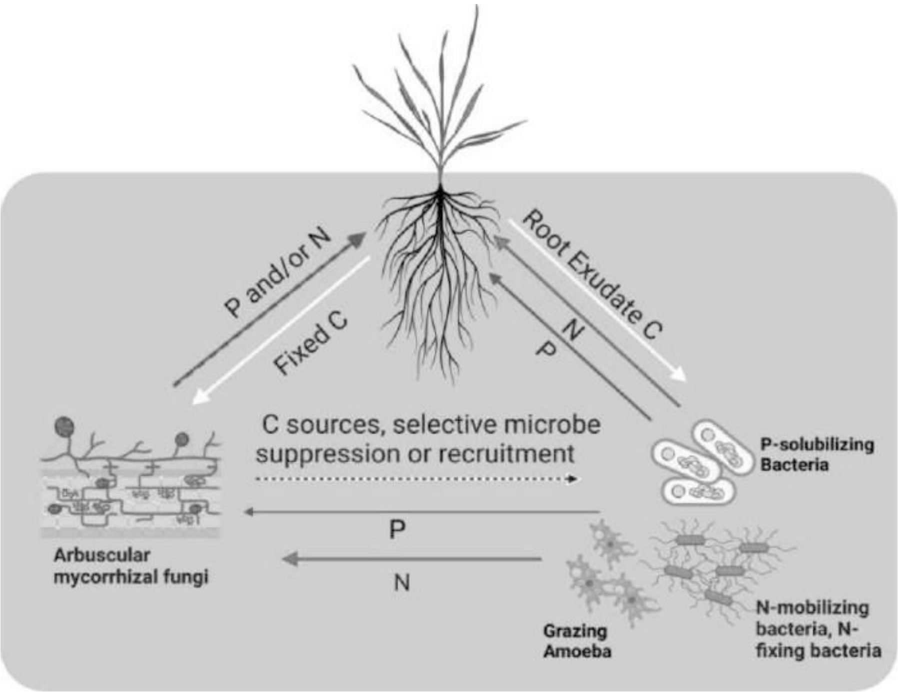


Fig : Activity of VAM in relation to other microbes

There are several hypotheses put forward to explain the mechanisms of plant disease control by mycorrhizal fungi, some are listed below :

(a) Thickening of cell wall through lignification and production of other polysaccharides which in turn hinder the entry of root pathogen (Dehne and Schoenbeck, 1979)

(b) Stimulation of the host roots to produce and accumulate sufficient concentration of metabolites (terpenes, phenols etc.), which impart resistance to the host tissue against pathogen invasion (Krupa et al., 1973; Sampangi, 1989)

(c) Producing antifungal and antibacterial antibiotics (Marx, 1972)

(d) Competing with the pathogens for the uptake of essential nutrients in the rhizosphere and at the roots surface (Reid, 1990)

(e) Stimulating the microbial activity and competitions in the roots and thus preventing the pathogen to get access to the roots (Rambelli, 1973).

(f) VAM can inhibit Zinc and Manganese uptake at toxic concentration in soil thus reducing adverse effect on host.

The beneficial microorganisms (antagonistic bacteria) (e.g., *Pseudomonas fluorescens, Bacillus subtilis*, etc.) and fungi (e.g., AMF, *Trichoderma,* etc.) compete with plant pathogens for nutrients and space, by producing antibiotics, by parasitizing pathogens, or by inducing resistance in the host plants, these microbes have been used for bio-control of pathogens.

Other uses

Increased rootlet size and longevity : Mycorrhizal plants have larger roots than non-mycorrhizal plants regardless of whether mycorrhizal fungi are present.

Tolerant of harsh conditions : Fungi are more tolerant of acidity, elemental toxicity and high soil temperatures than are higher plants and able to, in some cases they shield the roots from these condition. Lower levels of heavy metals generally found in mycorrhizal plants than non-mycorrhizal plants.

Increased seedling survival : Mycorrhiza promotes plant survival, whether new seedlings or out-planted container stock. Survival of inoculated plants can be up to five times the survival of un-inoculated plants. Improved survival is no doubt due to a combination of mycorrhizal benefits, including faster growth to help overtop weeds, protection from pathogens, and improved drought tolerance.

Isolation of Vesicular Arbuscular Mycorrhiza Species from Plant Roots

Sample collection :

The collected samples were taken in sealed plastic bags, labelled and transported to the laboratory in an insulated container. Before processing, all the samples were sieved (< 2 mm mesh size) to remove stones, coarse roots and other litter, and fine roots were collected from each sample. Soil samples were air-dried and stored at 4^0C for further experiments.

Isolation procedures :

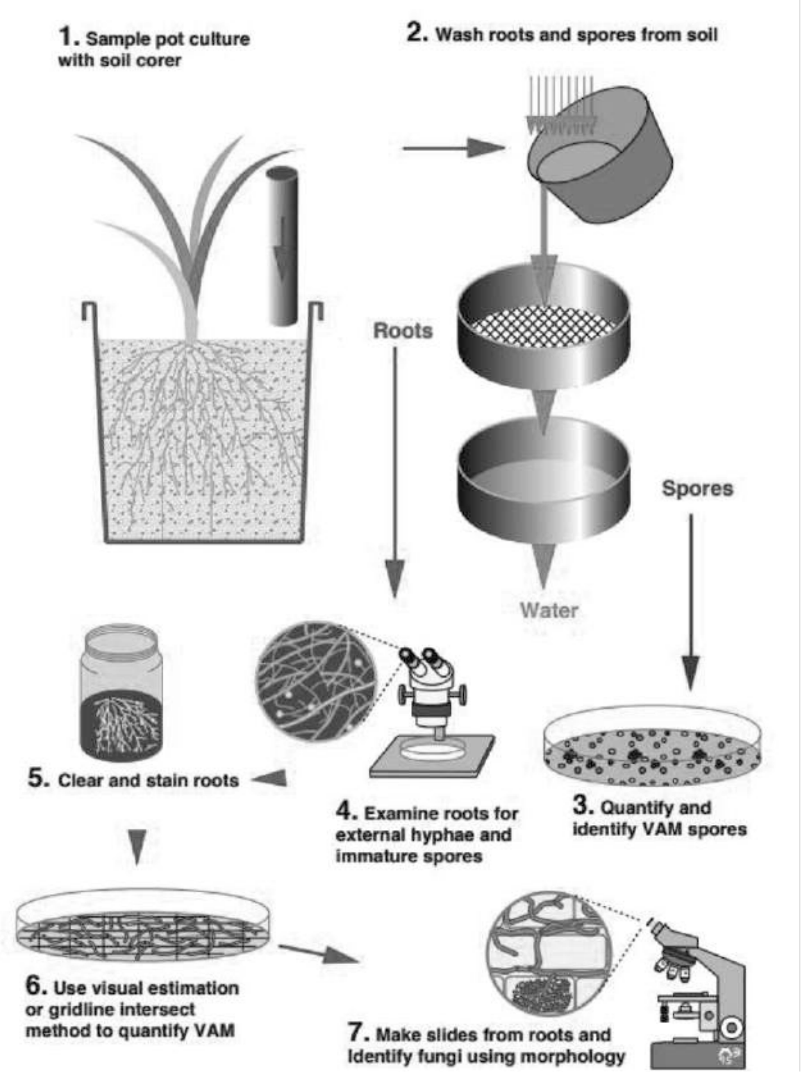
After sample collection next step was isolation of vesicular arbuscular mycorrhizal species collected from root samples.

The following steps were made :

1. Fine root samples were collected and then washed with running tap water and fixed in FAA (Formalin Acetic acid).

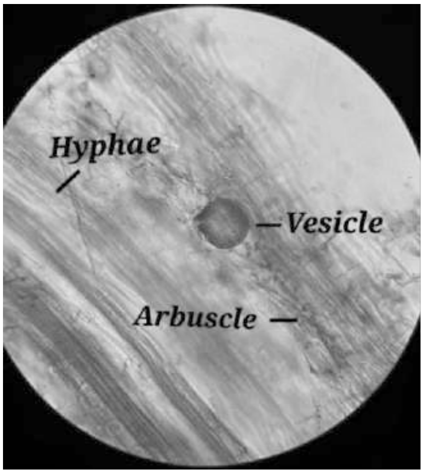
2. Roots were segmented into 1cm bits. Three replicates of 100 root bits each, selected at random were processed separately for determining the mycorrhizal intensity in the roots.
3. Root bits were treated with 10% KOH solution for 30 min at 40ºC temperatures. The concentration of KOH and time of incubation of roots depend upon the age and softness of the roots. Pour off the KOH solution and rinse the roots well in a beaker using at least three complete changes of tap-water or until no brown colour appears in the rinse water.
4. After thorough washing, root bits were stained with Trypan blue (0.01% trypan blue) for 24hrs at room temperature. Stained root pieces were mounted in lactoglycerol and examined under microscope for the mycorrhizal colonization.

SAMPLING VAM FUNGUS POT CULTURES



Observation :

Blue stained microscopic structures of tree-like arbuscles and balloon-like vesicles were observed. Hence, suggesting the mycorrhizal association by the penetration of hyphal parts into the roots of the plant body.



Photograph of microscopic field showing VAM (taken during practical sessions)

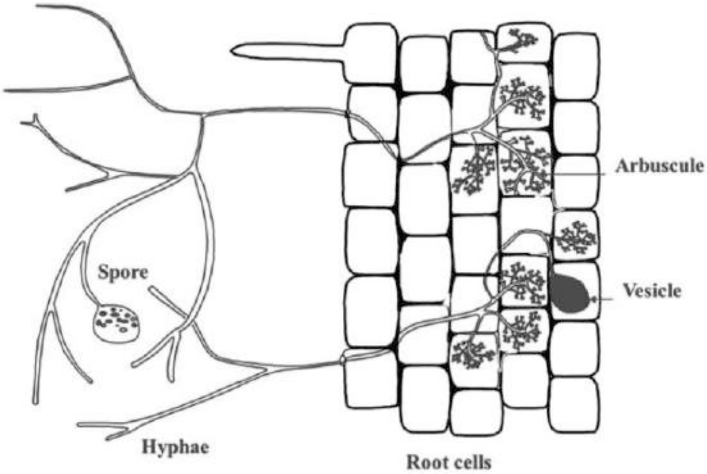


Fig : Diagrammatic representation of Vesicular Arbuscular Mycorrhiza

Conclusion

The amount of land used for crop production is decreasing daily as a result of rising input costs, particularly those of fertilizer. Due to the continuous use of fertilizer, we are in a position to reduce the use of inorganic fertilizer and increase the efficiency of applied nutrients. This is where VAM is employed.

VAM fungi offer a wide range of benefits for sustainable agriculture, environmental restoration, and ornamental plant cultivation. They can easily be applied to seeds, seedlings or soil and is a valuable tool to improve degraded lands. With proper understanding, management and strategic application, these beneficial fungi can promote healthier plants, improve soil quality, and reduce our reliance on harmful chemical inputs

Market available products for VAM inoculation

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STEM CELL THERAPY

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Semester V

Introduction

Stem cell therapy is an advanced medical treatment that involves using stem cells to repair or replace damaged tissues in the body. Stem cells have the unique ability to develop into different types of cells, which makes them valuable for treating a range of diseases and injuries.

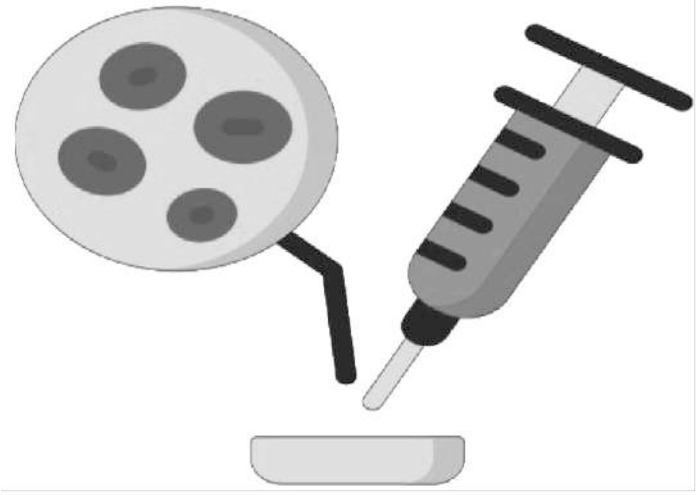


Fig: Stem cells therapy

Types of Stem Cells Used

1. **Embryonic Stem Cells (ESCs) :** Derived from embryos, these cells can turn into any cell type in the body. Their use is somewhat controversial due to ethical considerations.
2. **Adult Stem Cells :** Found in bone marrow, fat, and other tissues, these cells are limited in their ability to transform but are commonly used in therapies.
3. **Induced Pluripotent Stem Cells (iPSCs) :** Created in a lab from adult cells, iPSCs behave like embryonic stem cells and hold potential without the ethical issues.

How Stem Cell Therapy Works

In treatment, stem cells are injected or surgically placed in the area needing repair, like damaged tissue in the heart, joints, or spinal cord. Once there, the cells may help regenerate tissue, reduce inflammation, or modulate the immune response, depending on the condition being treated.

Conditions Treated with Stem Cell Therapy

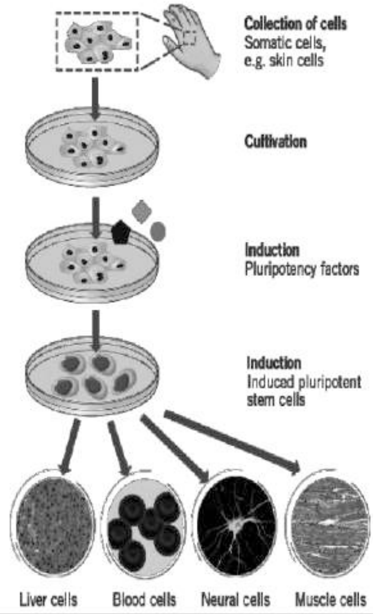
Stem cell therapy is currently being researched or applied to :

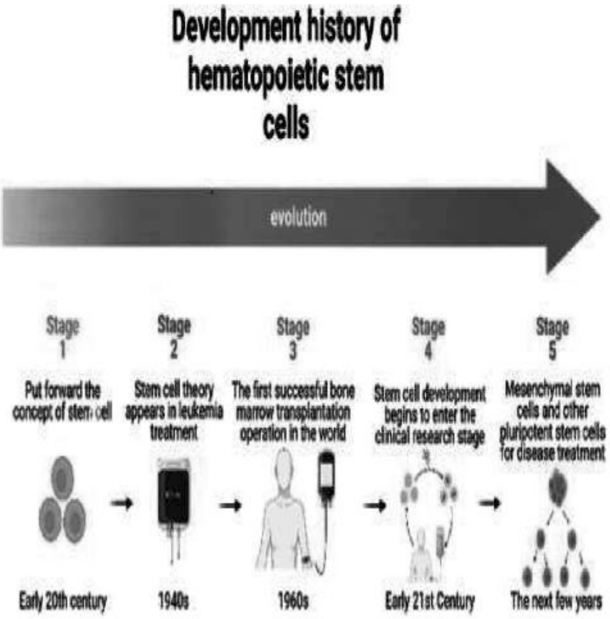
- Joint problems like osteoarthritis

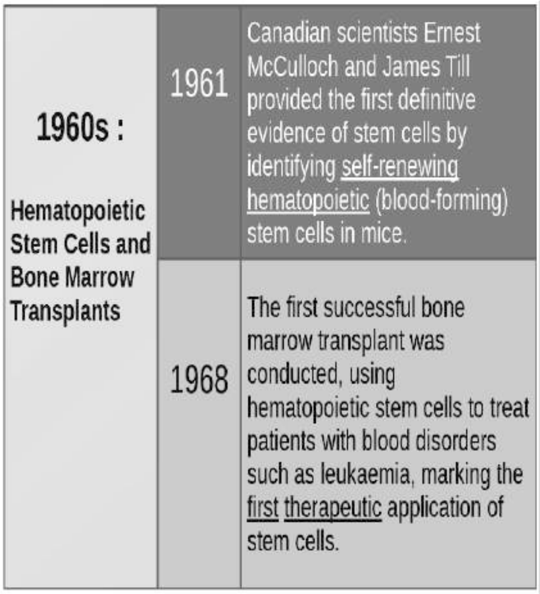
- Autoimmune diseases such as multiple sclerosis
- Neurological disorders like Parkinson's or spinal cord injuries
- Heart disease by helping to regenerate damaged heart tissue

History of Stem Cell Therapy

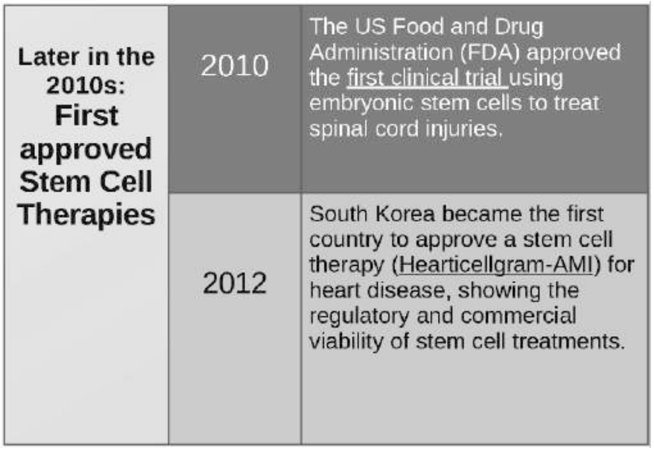
Early 20th century: **Stem Cell discovery**	1908	Russian histologist Alexander Maksimov introduced the concept of stem cells, hypothesizing the existence of undifferentiated cells that could produce blood cells.
	1950s	Researchers began understanding cell differentiation and discovered that certain cells could give rise to various tissue types, laying the foundation for stem cell research.



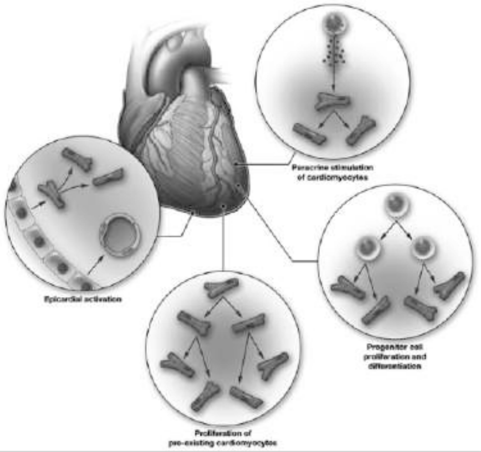


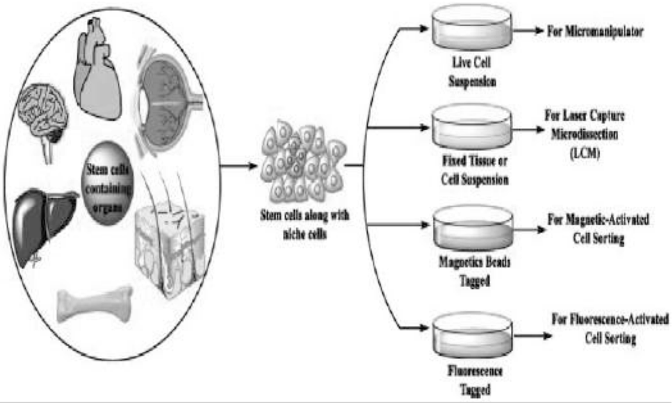


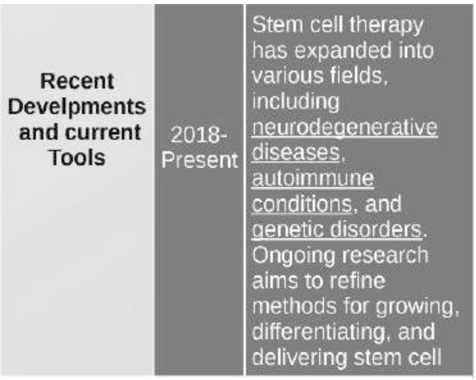
1960s : **Hematopoietic Stem Cells and Bone Marrow Transplants**	1961	Canadian scientists Ernest McCulloch and James Till provided the first definitive evidence of stem cells by identifying self-renewing hematopoietic (blood-forming) stem cells in mice.
	1968	The first successful bone marrow transplant was conducted, using hematopoietic stem cells to treat patients with blood disorders such as leukaemia, marking the first therapeutic application of stem cells.



Later in the 2010s: First approved Stem Cell Therapies	2010	The US Food and Drug Administration (FDA) approved the first clinical trial using embryonic stem cells to treat spinal cord injuries.
	2012	South Korea became the first country to approve a stem cell therapy (Hearticellgram-AMI) for heart disease, showing the regulatory and commercial viability of stem cell treatments.







Recent Develpments and current Tools	2018-Present	Stem cell therapy has expanded into various fields, including neurodegenerative diseases, autoimmune conditions, and genetic disorders. Ongoing research aims to refine methods for growing, differentiating, and delivering stem cell

ADVANTAGES OF STEM CELL THERAPY

- **Tissue Regeneration :** Repairs damaged organs and tissues.
- **Chronic Disease Treatment :** Potentially manages diseases like diabetes, Parkinson's, and heart disease.
- **Reduced Organ Transplant Need :** May decrease dependence on donor organs.
- **Pain Management & Faster Recovery :** Especially helpful in orthopaedic injuries.
- **Personalized Medicine :** Lowered risk of rejection when using patient's cells.
- **Advances in Drug Testing :** Better accuracy without animal testing.

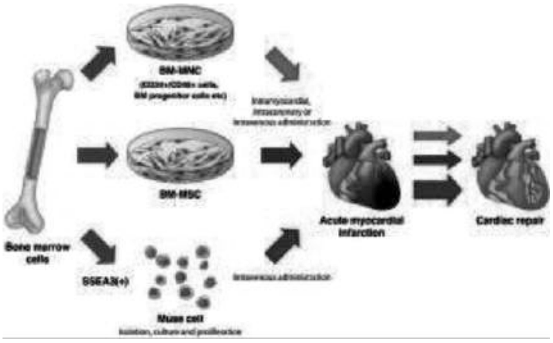
DISADVANTAGES OF STEM CELL THERAPY

- **Ethical Concerns :** Especially regarding embryonic stem cells.
- **High Cost :** Expensive and not always covered by insurance.
- **Risk of Tumour Formation :** Certain types of stem cells can grow uncontrollably.
- **Immune Rejection :** Possible if using non-autologous (donor) cells.
- **Limited Research on Long-term Effects :** Effects over time are not fully understood.
- **Complex Regulatory Issues :** Strict regulations can limit availability.

CASE STUDIES AND IT'S IMPACT

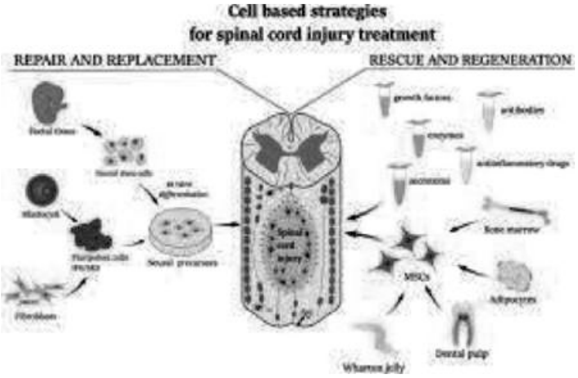
Stem cell therapy has shown promising results in various medical fields, including regenerative medicine, treatment of degenerative diseases, and even in certain cancers. Here are a few notable case studies that demonstrate the potential of stem cell therapy :

- **Cardiac Repair after Myocardial Infarction**



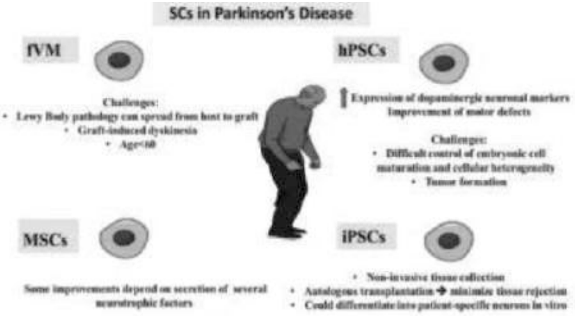
- ➢ **Case Study :** Patients with damaged heart tissue after myocardial infarction (heart attack) have been treated with stem cell therapy to promote heart tissue regeneration.
- ❖ **Therapy :** Mesenchymal stem cells (MSCs) or induced pluripotent stem cells (iPSCs) are injected into damaged heart tissue.
- ❖ **Results :** Studies have shown improvement in cardiac function, with some patients experiencing reduced scarring and enhanced heart function. Long-term results are still under observation, but early results indicate that stem cell therapy may reduce the risk of heart failure following a heart attack.

- **Spinal Cord Injury Treatment**



- ➢ **Case Study :** A clinical trial led by researchers used human neural stem cells in patients with chronic spinal cord injuries to assess nerve regeneration.
- ❖ **Therapy :** Human neural stem cells were implanted in the damaged areas of the spinal cord to regenerate neurons and improve motor and sensory functions.
- ❖ **Results :** Several patients regained partial motor functions, with some showing improved sensory perception and muscle control. While not all patients had the same degree of recovery, this study highlighted the potential for stem cell therapy to aid spinal cord injury repair.

- **Parkinson's Disease**



- ➢ **Case :** In 2017, an Australian man with Parkinson's disease participated in a stem cell therapy trial.
- ❖ **Treatment :** Neural stem cells were transplanted into the patient's brain to replenish dopamine-producing cells that had been lost due to Parkinson's.

- **Outcome :** The patient reported improvements in motor function and a reduction in symptoms, although long-term effects and safety continue to be monitored.

The greatest impact of stem cells therapy is that it can generate healthy cells to replace cells affected by disease. Stem cells can be guided into becoming specific cells that can be used in people to regenerate and repair tissues that have been damaged or affected by disease.

CONCLUSION

It's essential to recognise both its immense potential and its current limitations. Stem cell therapy holds promise for regenerating damaged tissues, treating chronic diseases and even reversing degenerative conditions making it one of the most exciting areas on modern medicines.

However, while promising, stem cell therapy is still in its developmental stages for many applications. The ethical conditions, regulatory challenges, and the risk of potential complications such as immune rejections or uncontrolled cell growth highlight the need for careful oversight.

Stem cell therapy is a transformative frontier in medicine with the potential to revolutionize healthcare. However, to harness its full benefits, a balance between innovation, regulation and ethical consideration is essential.

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ARTIFICIAL INTELLIGENCE AND SOCIETY

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Semester V

Artificial intelligence (AI) is a branch of computer science that aims to create machines that can perform tasks that would usually require human intelligence. It is an evolving technology that tries to simulate human intelligence using machines.Today, AI systems are used to support or replace humans in everyday life, and are associated with an increasing automation of social processes.

Artificial intelligence, once confined to the realms of science fiction, has seamlessly woven itself into the fabric of our society. From the mundane tasks of our daily lives to the groundbreaking discoveries that shape our world, AI's influence is undeniable.

THE DAWN OF AI :

The journey of AI began in the mid-20th century, with pioneers like Alan Turing laying the foundation for intelligent machines. Early AI systems focused on logical reasoning and problem-solving, mimicking human cognitive abilities. However, it was the advent of machine learning and deep learning in recent decades that truly unleashed AI's power. Artificial Intelligence is a technology that originated since the early 1950's. But it wasn't until November 2022 that it went mainstream. Open AI, an AI-focused tech company released version 1 of ChatGPT and the internet went wild. Open AI ended up having 1 million users in less than a week and it created a stir in the world.

POSITIVE IMPACTS OF AI IN SOCIETY :

- **Improved decision-making**

  AI can analyze large amounts of data to help make better decisions and predictions. This can be useful in many fields, including finance, healthcare, and climate science.

- **Reduced human error**

  AI can perform tasks more consistently than humans, especially repetitive or dangerous tasks. For example, robots can be used in areas with high radiation where humans could be seriously harmed.

- **Improved healthcare**

  AI can help diagnose diseases and develop personalized treatment plans. AI can also be used to analyze medical imagery and data.

- **Improved transportation**

  Self-driving cars and other autonomous vehicles can reduce traffic accidents and improve transportation efficiency.

- **Improved communication**

  AI-powered language processing can improve language translation, speech recognition, and chatbot interactions.

- **Improved environmental protection**

  AI can be used to detect poachers and fast-moving weather patterns. AI can also be used to design new proteins that can eat plastic and radioactive materials.

- **Improved creativity**

  AI can help enhance creativity and improve productivity.

- **Improved wellness**

  AI can help generate ideas for wellness interventions, such as gratitude, kindness, or fun activities.

NEGATIVE IMPACTS OF AI IN SOCIETY :

- **Job Displacement**

AI systems can automate many tasks, leading to job losses in various sectors like manufacturing, customer service, and even some creative fields. This can have significant economic and social implications. AI systems are currently limited in their ability to think creatively and understand human emotions. While they can process vast amounts of data and make predictions, they struggle with tasks that require empathy, intuition, and original thought.

- **Ethical Concerns and Bias**

AI algorithms can perpetuate existing biases present in the data they are trained on. This can lead to discriminatory outcomes, especially in areas like hiring, lending, and criminal justice. The development and use of AI raise ethical questions about accountability, transparency, and the potential for misuse.

- **High Costs**

Developing and implementing AI systems can be expensive, requiring significant investments in hardware, software, and specialized expertise. This can limit access for smaller organizations and individuals.

- Lack of Common Sense and Contextual Understanding

AI systems often struggle with understanding nuances, context, and common sense. They can be easily misled by ambiguous or incomplete information. This can lead to errors and misinterpretations, especially in complex situations.

FEATURES OF AI SYSTEM THAT SHOULD BE ENCOURAGED :

Encouraging specific features in AI systems can lead to more ethical, reliable, and useful outcomes. Here are some essential AI features to promote :

1. **Transparency and Explainability :** AI systems should be able to explain their decision-making processes, making it clear how they reach conclusions. This helps users understand and trust AI, especially in critical applications like healthcare or finance.
2. **Fairness and Bias Mitigation :** AI should be designed to avoid discrimination or bias. Diverse training data and bias-checking processes can help ensure decisions are fair and equitable across demographics.

3. **Data Privacy and Security :** Protecting user data is crucial. AI systems should follow stringent data protection standards, including anonymization, encryption, and user-consent protocols.
4. **Accountability :** There should be clear responsibility for an AI system's actions. Having accountable design, usage policies, and oversight structures helps ensure responsible AI use.
5. **Robustness and Safety :** AI systems should be designed to perform reliably across diverse situations, with mechanisms to handle unexpected inputs and avoid harmful behavior.
6. **User-Centered Design :** AI should prioritize user needs, allowing for easy interaction, customization, and feedback mechanisms to refine performance.
7. **Ethical and Responsible Behavior :** Systems should be designed to align with ethical guidelines and avoid actions that could harm individuals or society.
8. **Continuous Learning and Adaptation :** Encouraging AI to adapt and improve over time, while ensuring that updates are safe and properly validated.
9. **Environmental Responsibility :** Efficiency in computational resource use helps reduce the environmental impact, especially for large-scale models.
10. **Collaboration and Augmentation :** AI should be developed as a tool to enhance human abilities, supporting collaborative use cases and amplifying productivity in ethical ways.

Promoting these features in AI development can drive systems that are not only powerful but also aligned with societal values and user needs.

POLICIES FOR MANAGING AND INTEGRATING AI DEVELOPMENT IN SOCIETY :

AI will have an important impact on a range of sectors in society, augmenting or replacing human work. The challenge is to anticipate these changes and develop policies that will limit negative effect sand allow a better integration of AI. Education is key both in driving AI adoption and in combating inequality. Basic understanding of the use of data and AI technologies is needed across all ages, not only of producers and professional users of AI but for all citizens. Introducing key concepts in schools can help ensure this. Adopting a broad and balanced curriculum for educating young people in sciences, mathematics, computing, arts and humanities could equip them with a range of skills and provide a stronger basis for lifelong learning. There is also high-demand for highly skilled employees. A range of sectors and professions will require skills to use AI in ways that are useful for them. New initiatives can help create a pool of informed users of AI systems. Support for novel apprenticeship tracks and infrastructures is also needed to build advanced skills in AI that will allow new applications with the creation of many new jobs. These issues were already part of the declaration of Ottawa on "Realizing our digital future and shaping its impact on knowledge, industry, and the workforce" at the last G7 summit. Governments are encouraged to implement policies that will be inclusive and able to provide every citizen with equitable access to the AI benefits. This requires that information quality, security and resilience are also guaranteed as well as transparency, openness and interoperability of the AI systems. In those areas where AI's capabilities have outpaced current regulations, there may be a need for new governance approaches that take into account ethical questions arising from human interaction with intelligent machines. It is worth emphasizing the role of humanities and social sciences broadly and in partnership with developers and users in exploring the ways in which AI may challenge existing ethical norms or indeed reveal the ways in which AI presents new ethical challenges.

USE OF AI IN DIFFERENT FIELDS :

AI in different fields is expected to add $15.7 trillion to the global economy, which is more than the current economies of China and India make. Most of the gains will be provided by AI-based product enhancements that boost consumer demand. It becomes possible due to better personalization and affordability of products and services backed up with artificial intelligence.

- **AI in banking and financial services**

  Known as slow to adopt new technologies, traditional brick-and-mortar banks are now facing the challenge of competition with tech-driven, super flexible, and digital-only fintechs. However, some of them are building successful digital transformation strategies that provide better online user experience, implement data analytics and automate services to provide more competitive pricing.

- **AI in healthcare**

  Disease prediction with the help of statistical data – AI allows diagnosis and treatment of health issues at earlier stages due to the patient's medical history analysis. The implementation of this type of AI in the industry of healthcare allows for timely prevention, decreases therapy costs, and timely delivery of individual recovery plans.

  Risk assessment – AI-based decision-making systems implement the capabilities of neural networks and machine learning to assist medical personnel in the evaluation of risks and success in treatment.

  Digital healthcare tools – AI in healthcare apps helps with diagnosis chatbots and data monitoring to alert potentially dangerous health conditions. Also, it includes Natural Language Programming that translates written or spoken notes into actionable patient data.

- **AI in agriculture**

  The agriculture industry may implement AI for computer vision technology to monitor soil and crop in order to yield healthier crops, control soil erosion, support necessary conditions for plants and make farming less effort-consuming in general.

  With Gini Machine farmers can upload historical data and build predictive models to assess price and demand fluctuations.

- **Travel and hospitality**

  In addition to face recognition technologies in airports, there are more AI-based services appearing for private companies to make traveling more affordable and pleasant. For example, AI in travel booking and flight price prediction, customer service AI in airports and hotels that use robo-assistants, and more. More specific AI-based apps include AI for travel disruption management that suggest alternative solutions in case of bad weather or other delays. Just like many other industries, travel and hospitality may benefit from customer churn prediction with the help of AI.

AI data analytics in hospitality is widely used in luxury hotels and resorts. Analyzing visitors' sentiments, the system may improve guest experience, timely reveal problems, and send notifications about the necessity of human intervention.

THE FUTURE OF AI AND SOCIETY :

The future of AI holds immense promise, but it also presents challenges. To harness the benefits of AI while mitigating its risks, a collaborative approach is needed involving policymakers, technologists, ethicists, and society as a whole. By fostering responsible AI development and ethical guidelines, we can ensure that AI serves as a force for good, enhancing human capabilities and improving the quality of life for all.

The positive impact of artificial intelligence on society is undeniably profound, touching various aspects of our lives and shaping a future that was once only imaginable in science fiction. From advancements in healthcare, where AI aids in early detection and diagnosis, to the optimisation of business processes through automation, and even the transformation of education with personalized learning, AI has proven to be a catalyst for positive change.

While ethical considerations and responsible AI development are crucial aspects, the overall trajectory of AI's positive impact on society signifies a promising era of technological advancement, improved quality of life, and increased accessibility to solutions that benefit humanity. However, the use of AI also raises questions about human values, fairness, privacy, and accountability. As AI systems become more prevalent, it's important to consider how they will impact society and how to ensure they are used responsibly.

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EXPLORING DE-NOVO MUTATIONS TO ANTIMICROBIAL RESISTANCE

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The increased usage of antibiotics has led to the emergence of bacteria resistant to many last-resort antibiotics widely used in healthcare settings. In addition to this, no new class of antibiotics has been discovered in the past 40 years, leading to the rise of multi-drug-resistant pathogens. While some alternative approaches to antibiotics involve antibiotic combinations, reports from previous literature as well as our lab have identified clinical isolates that exhibit resistance to beta-lactam and beta-lactamase inhibitor combinations, such as piperacillin-tazobactam and ceftazidime-avibactam. While much is known about the plasmid-borne resistance mechanisms, a lot is yet to be discovered regarding genomic changes that can confer, or enhance resistance. To address this gap in the knowledge we first looked at the genetic determinants of the resistance to the two aforementioned beta lactam-beta lactamase inhibitor combinations in thirty-three *Escherichia coli* clinical isolates. All the prominent genes predicted to confer resistance belonged to the beta lactamase categories, which are known to be transferred by horizontal gene transfer method. In the next step, we evolved replicate *E. coli* K12 MG1655 populations in these two, and another beta lactam-beta lactamase inhibitor combination (aztreonam-avibactam). For this, we started with determining permissible concentrations and the minimum inhibitory concentrations (MICs) of each drug for *E. coli*. We then exposed these populations to laboratory-based directional evolution experiments over approximately 81 generations, starting from the permissible concentrations and escalating to the MICs. The fitness of the evolved populations were then assessed and compared to that of the ancestor in antibiotic-free as well as antibiotic containing environments.

We are currently investigating the genetic mechanisms underlying phenotypic resistance in evolved bacterial populations through both clone and whole-population sequencing. The identification of de-novo mutations would reveal the intrinsic mechanisms of resistance in bacterial populations in response to antibiotic exposure. This would allow us to cross-reference the clinical data, investigating the presence of similar polymorphisms in resistant isolates. Our findings would, in short, potentially guide the development of novel therapeutic strategies.

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CRISPR/CAS9 GENOME EDITING TECHNOLOGY FOR THE IMPROVEMENT OF CROPS

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Semester V

KEYWORDS

CRISPR/Cas9, Genome editing, Climate change, Blast disease, Genetic engineering, Food security

ABSTRACT

With the growing population, it is expected that the number will significantly grow from 7.3 billion to 9.7 billion by the year 2050. Increased abiotic stress like drought, salinity, high temperature, and outbreaks and losses caused by pests, all due to the consequential climate change, pose a high risk to the production of crops and are a threatening danger to food security all over the world. Although conventional breeding techniques such as hybridization and mutation breeding etc, have increased the yield of crop production the global demand for food has notably risen. Hence new approaches are required to improve the production of crops to meet the increasing global demand. For rapidly emerging challenges in this field of agriculture, a highly efficient and precise, The Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)/Cas9 genome editing technology has been introduced. It can be used to precisely modify the genome sequence of any organism including plants to achieve the desired trait with the help of a guide RNA and a protein. To ensure the future food security of the expanding population, and to prevent them from harmful effects CRISPR/Cas9 has become a feasible tool for the growth of non-transgenic genome-edited crop plants. This review will discuss the potential of CRISPR/Cas9 for the improvement of different cultivated crops to gain toughness against emerging pests and abiotic stresses.

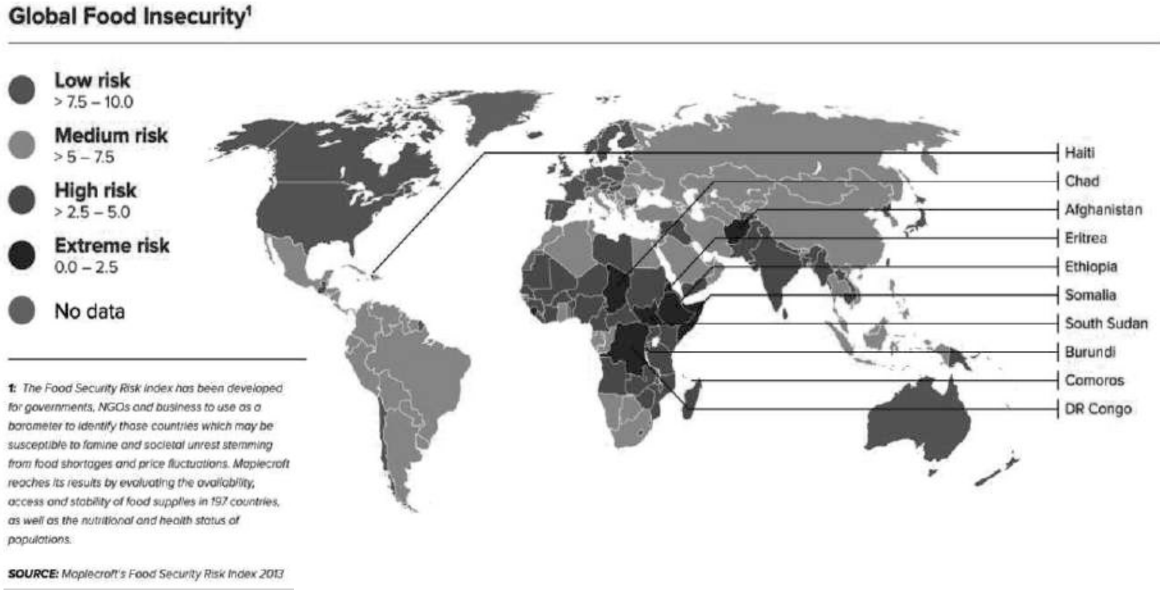
INTRODUCTION

Some of the alarming issues circulating in the world community in the current times are climate change, population growth, and shortage of food. Population around the globe is increasing rapidly and is estimated to reach 9.7 billion by the year 2050. Considering the population growth, the rate of it is higher in the tropical region than in the temperate region. Countries like India, Nigeria, the Democratic Republic of the Congo, Ethiopia, Nigeria, the Republic of Tanzania, Indonesia, and Uganda are estimated to show a spike in the charts while Nigeria is the largest among all the countries to show a rise in the population growth after China. With this briskly growing population, the critical issue of providing food to everyone will need to be addressed by society. With the growing population, the availability of arable land is getting reduced day by day which is also leading to reduced crop yields. For instance, the International Rice Research Institute (IRRI) has estimated that one hectare of cultivable land is lost every 7.7 s, and the rate of loss may accelerate with increased global temperatures (Stamm et al., 2011). Climate change brings about adverse stresses in the environment like altered weather patterns, soil salinity, drought, flood, and emergence of new pests and diseases. To cope with the rising population and to have a well-fed society, the production of food is estimated to grow 50% by 2030 to 70-100% by 2050(Godfray et al., 2010; Jones et al., 2014). To meet the call of this growing demand on an urgent basis, crops of better varieties with high adaptability to this changing climate as well as more tolerant to the biotic and abiotic stress need to be introduced.

Techniques of conventional breeding like hybridization, mutation breeding, backcrossing, etc enhances the potential of the crop yield still the actual crop yield seems to be approaching a plateau in recent decades (Mann, 1999; Ansari et al., 2017). Crops like rice in East Asia, maize in South Europe, and wheat in Northwest Europe, have seen a yield stagnation of these plants. This is threatening to the world's extensive cropping system.

The Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)/CRISPR-associated protein (Cas9) genome editing technology (CRISPR/Cas9) is found to be a useful tool for the improvement of crops and has shown significant wins in addressing the different challenges in the field of agriculture. It is a genome editing technology that is sequence-specific and with the availability of PAM(Protospacer Adjacent Motif), it modifies sequences to achieve desired traits in organisms including plants. In regards to other genome editing tools like Meganucleases and Zinc Finger Nucleases (ZFNs), CRISPR/Cas9 is a cost-effective, easy-to-use, highly efficient, and precise tool used for genome editing.

review aims to present updated information on the application of CRISPR/Cas9 technology for improving various crops, particularly those grown in tropical climates like rice and wheat. It emphasizes how CRISPR/Cas9 can enhance crop resilience to changing climate conditions, combat emerging diseases, and improve product quality, addressing specific challenges faced by tropical crops.



Global Food Insecurity[1]

1: The Food Security Risk Index has been developed for governments, NGOs and business to use as a barometer to identify those countries which may be susceptible to famine and societal unrest stemming from food shortages and price fluctuations. Maplecroft reaches its results by evaluating the availability, access and stability of food supplies in 197 countries, as well as the nutritional and health status of populations.

SOURCE: *Maplecroft's Food Security Risk Index 2013*

Fig: The map provides a visualization of the extent to which food insecurity affects the world

CRISPR/Cas9

The CRISPR-Cas9 system is a potent genome editing tool that originates from the adaptive immune systems of prokaryotes. Its mechanism consists of three primary steps: recognition, cleavage, and repair (Mengstie & Wondimu, 2021). The system includes two key components: a guide RNA (gRNA) and the Cas9 endonuclease protein. During the recognition phase, the designed single guide RNA (sgRNA) identifies and binds to the target DNA sequence through complementary base pairing. The Cas9 nuclease then cuts the DNA at a location three base pairs upstream of the protospacer adjacent motif (PAM), resulting in a double-stranded break (Mengstie & Wondimu, 2021; Zhang et al., 2014). For CRISPR/Cas9 to effectively cleave DNA, the target sequences must match the crRNA and a PAM must be present downstream of these sequences. After cleavage, the cell's DNA repair mechanisms are triggered. The double-stranded break can be repaired via either non-homologous end joining (NHEJ) or homology-directed repair (HDR) (Mengstie & Wondimu, 2021). These repair processes can yield desired insertions, deletions, or substitutions at the target sites, enabling precise genome editing (Zhang et al., 2014).

Interestingly, the original role of the CRISPR-Cas9 system in prokaryotes was to serve as an adaptive immune defense against phages and plasmids (Bhushan, 2020). The system retains memories of previous infections by incorporating short segments of foreign DNA, known as spacers, into the CRISPR array through a process called adaptation (Sternberg et al., 2016). This illustrates the evolutionary background of the CRISPR-Cas9 system, which has emerged from the ongoing evolutionary battle between bacteria and phages (Bhushan, 2020).

The mechanism of action of the CRISPR-Cas9 system—comprising recognition, cleavage, and repair—has transformed genome editing across various domains, including medicine, agriculture, and biotechnology (Bhatia et al., 2023; Mengstie & Wondimu, 2021). Its simplicity, efficiency, and versatility have made it a favored tool among researchers, allowing for precise manipulation of specific genomic elements and aiding in the understanding of target gene functions in biology and disease (Cui et al., 2017; Zhang et al., 2014).

USE OF CRISPR/Cas9 in AGRICULTURE

The agricultural sector faces numerous challenges, including biodiversity loss, habitat destruction, climate change, water scarcity, excessive land use, and the indiscriminate application of chemical fertilizers and pesticides, along with food waste. Agriculture plays a crucial role in combating food insecurity, yet various obstacles hinder its capacity to ensure adequate food supply. Food insecurity remains a pressing issue, threatening millions globally due to factors such as population growth, urbanization, poverty, economic instability, and conflict. CRISPR/Cas9 has the potential to enhance food security by increasing crop yield and quality, improving nutritional value through biofortification, and minimizing post-harvest losses. This gene-editing technology can also help reduce greenhouse gas emissions by enhancing carbon capture.

Modification of Nutritional Content

CRISPR-Cas9 can be utilized to alter the nutritional profiles of crops, including seed grain weight, protein content, polyunsaturated fatty acid levels, mineral content, and amylopectin content. Unlike other genetically modified crops, CRISPR-Cas9 does not introduce foreign genes into the plant genome; instead, it targets specific genes responsible for nutrient biosynthesis to enhance their nutritional value. Specifically, it focuses on genes that regulate the production of bioactive compounds such as phenolics, carotenoids, vitamin E, dietary fiber, and beta-glucan. By manipulating these genes, researchers can boost the production of these beneficial compounds in crops, thereby improving their nutritional quality. This method has been successfully applied to biofortify various cereal and vegetable crops, including rice, wheat, and carrots. For instance, in one study, CRISPR-Cas9 was employed to target the FAD2 gene in *Camelina sativa*, resulting in a 16% to 50% increase in oleic acid content in the seeds.

Enhancing Produce Quality and Shelf Life :

CRISPR-Cas9 technology is advantageous for agriculture as it not only extends the shelf life of crops but also enhances their overall quality. In a notable study, researchers applied this gene-editing tool to improve the shelf life of the melon species *Cucumis melo*. They specifically targeted the CmACO1 gene, which plays a crucial role in the ethylene synthesis pathway and regulates the shelf life of melons. Ethylene, a plant hormone, is essential for fruit ripening and senescence. The CmACO1 gene encodes an enzyme known as ACC oxidase, which catalyzes the final step in the ethylene biosynthesis process. By modifying the CmACO1 gene, the production of ethylene in melons was reduced, leading to a significant increase in their shelf life. This improvement helps minimize produce loss and contributes to food security.

IMPROVING YIELDS AND QUALITY OF CROPS CULTIVATED USING CRISPR TECHNOLOGY

To address the challenges posed by climate change, scientists are employing CRISPR/Cas9 technology to engineer plants that can thrive in harsh and unpredictable environments while also increasing their yield. This innovative genome editing tool allows for precise modifications in the genetic makeup of plants, enabling researchers to enhance specific traits that contribute to better growth and productivity.

At Cold Spring Harbor Laboratory (CSHL), researchers utilized CRISPR/Cas9 to modify the promoter sequences of quantitative genes in tomatoes. These genes control various traits, such as fruit shape and size (LOCULE NUMBER), fruit size (FASCINATED), flower proliferation (COMPOUND INFLORESCENCE), and flowering time (SELF PRUNING). By making targeted changes to these promoter regions, the scientists were able to create new genetic variations (alleles) that improved the overall fruit shape, size, and plant architecture (Rodríguez-Leal et al., 2017). This approach is particularly valuable in situations where natural genetic variations are limited or have diminished over time.

In rice, researchers simultaneously targeted three genes (GW2, GW5, TGW6) that negatively regulate seed size. By mutating these genes using CRISPR/Cas9, they achieved a significant increase in seed size—up to 30% in the triple mutants. This demonstrates the potential of CRISPR/Cas9 to enhance crop yield by modifying key genetic factors.

Similarly, in *Brassica napus*, the mutation of CLVTA3 genes led to an increase in both the number of seeds per silique and the overall grain weight. In wheat, the same technology was applied to knock out the TaGW2 gene, which is known to negatively regulate seed size. This resulted in increased grain weight, width, and length, showcasing the effectiveness of CRISPR/Cas9 in improving crop yields (Wang W. et al., 2018).

Beyond yield improvements, CRISPR/Cas9 is also being used to enhance the quality of crops. For instance, researchers targeted phytoene desaturase genes in bananas, which are crucial for carotenoid biosynthesis—important for plant health and nutrition. The successful mutation of these genes with a 59% success rate indicates that CRISPR/Cas9 can be effectively used to improve the nutritional quality of crops (Kaur et al., 2018).

Overall, the application of CRISPR/Cas9 technology in agriculture holds great promise for developing crops that are not only more resilient to climate change but also higher in yield and quality, thereby contributing to food security in the future.

CRISPR/CAS9 GENOME EDITING FOR ADDRESSING EMERGING DISEASES AND PESTS IN CROPS CULTIVATED

The global food security is on a major threat. Pathogens like bacteria, fungi, viruses and oomycetes causes different crop diseases which results in the considerable pre harvest yield losses. The diseases are becoming frequent and posses an alarming danger to the crops. Despite advancement in the practices done while cultivation, the risk of yield losses is still estimated upto 15% of the food crops. While destruction caused by the pathogen is a significant threat, pest outbreak also contribute to the crop losses worldwide.

The CRISPR/Cas9 genome editing technology offers a promising solution for developing disease-resistant crop varieties. This technology allows for the rapid modification of plant genomes by either stacking disease-resistant genes (R-genes) or disrupting/deleting susceptibility genes (S-genes). Thus, this method would significantly enhances the ability of plants to fight and resist diseases and improves the quality of the crop yields.

Rice is a staple food and is consumed in large amount globally. It is vulnerable to many diseases and pests. Recently, a fungus, *Magnaporthe oryzae*, has caused blast disease in rice, which has caused a major destruction to the production of rice. The host has expanded itself to other crops like wheat, barley, millet and oat as well. The destruction caused is so big that could potentially feed millions and millions of people around the world. The factors which have influenced the rise of the blast disease were the ability of the pathogen to jump between grasses and other factors like climate change and global trade.

To combat this issue, rice lines with enhanced resistance to the blast disease have been introduced by the CRISPR/Cas9 genome editing technology. For instance, a deep study by Wang and his colleagues involved engineering a CRISPR/Cas9 vector to target the OsERF922 gene in rice, which regulates negatively to the plant's resistance to blast disease. By delivering this vector into rice calli derived from a blast-susceptible variety, they achieved a mutagenic frequency of 42% in the transgenic plants. Thus, the edited lines resulted in significant improved resistance to blast disease compared to the wild-type plants.

In recent times, CRISPR/Cas9 has been used to disrupt susceptibility genes which were associated with bacterial blight in rice, such as OsSWEET11 and OsSWEET14. In another study, it was shown that destroying the OsSEC3A gene in rice, has shown better resistance to blast pathogen and in better defense responses. These examples show the great potential of the CRISPR/Cas9 technology to improve plant immunity and resistance against crop diseases, thus protecting and enhancing the security of the food.

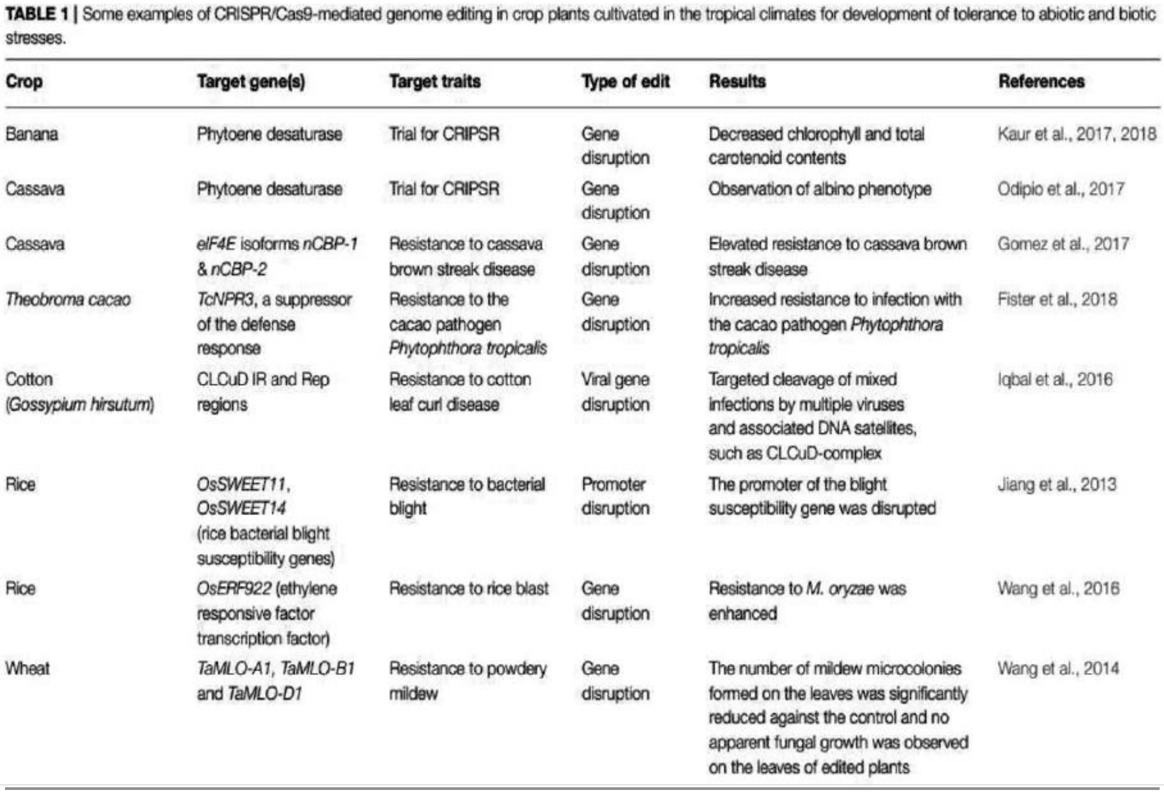


TABLE 1 | Some examples of CRISPR/Cas9-mediated genome editing in crop plants cultivated in the tropical climates for development of tolerance to abiotic and biotic stresses.

Crop	Target gene(s)	Target traits	Type of edit	Results	References
Banana	Phytoene desaturase	Trial for CRIPSR	Gene disruption	Decreased chlorophyll and total carotenoid contents	Kaur et al., 2017, 2018
Cassava	Phytoene desaturase	Trial for CRIPSR	Gene disruption	Observation of albino phenotype	Odipio et al., 2017
Cassava	*eIF4E* isoforms *nCBP-1* & *nCBP-2*	Resistance to cassava brown streak disease	Gene disruption	Elevated resistance to cassava brown streak disease	Gomez et al., 2017
Theobroma cacao	*TcNPR3*, a suppressor of the defense response	Resistance to the cacao pathogen *Phytophthora tropicalis*	Gene disruption	Increased resistance to infection with the cacao pathogen *Phytophthora tropicalis*	Fister et al., 2018
Cotton (*Gossypium hirsutum*)	CLCuD IR and Rep regions	Resistance to cotton leaf curl disease	Viral gene disruption	Targeted cleavage of mixed infections by multiple viruses and associated DNA satellites, such as CLCuD-complex	Iqbal et al., 2016
Rice	*OsSWEET11*, *OsSWEET14* (rice bacterial blight susceptibility genes)	Resistance to bacterial blight	Promoter disruption	The promoter of the blight susceptibility gene was disrupted	Jiang et al., 2013
Rice	*OsERF922* (ethylene responsive factor transcription factor)	Resistance to rice blast	Gene disruption	Resistance to *M. oryzae* was enhanced	Wang et al., 2016
Wheat	*TaMLO-A1*, *TaMLO-B1* and *TaMLO-D1*	Resistance to powdery mildew	Gene disruption	The number of mildew microcolonies formed on the leaves was significantly reduced against the control and no apparent fungal growth was observed on the leaves of edited plants	Wang et al., 2014

Fig: Some examples of CRISPR/Cas9-mediated genome editing in crop plants for development of tolerance to abiotic and biotic stresses

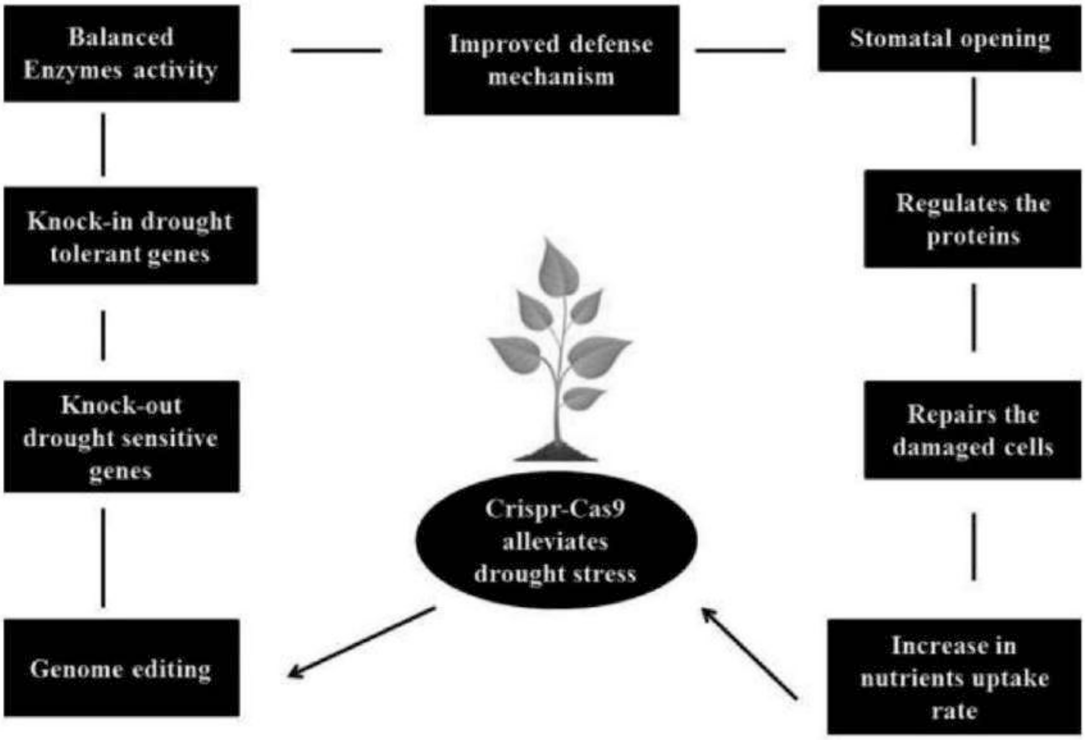
CRISPR-based Targeted Genome Editing for Drought Tolerance

Various abiotic stresses, such as drought, significantly impact crop yield by inhibiting plant growth and reducing overall productivity. Drought stress, in particular, is dynamic and can vary in intensity and duration, making it a challenging factor for crop resilience. Historically, genomic adaptation has been the primary method to achieve drought tolerance in plants. This involves the overexpression of specific genes and transcription factors that are associated with drought signaling pathways. These genes help in the accumulation of signaling molecules and metabolites that enhance the plant's ability to withstand drought conditions.

However, certain sensitive (S) genes can increase a plant's susceptibility to drought. The expression of these S genes can lead to hormonal imbalances, reduced antioxidant activity, and increased production of reactive oxygen species (ROS), which can be detrimental to plant health. For instance, proteins such as OsSRFP1, OsDIS1, and OsDST act as negative regulators of drought tolerance. Silencing these genes has been shown to elevate antioxidant enzyme levels, decrease hydrogen peroxide (H2O2) concentrations, and ultimately improve drought tolerance in rice.

A significant advancement in this area is the application of the CRISPR-Cas9 system to introduce specific mutations in genes that regulate drought response. One notable example is the modification of the OPEN STOMATA 2 (OST2) gene in *Arabidopsis*, which encodes a key plasma membrane H+ ATPase involved in stomatal function. Stomata are small openings on the leaf surface that regulate gas exchange and water loss. Under drought conditions, the plant hormone abscisic acid (ABA) binds to the OST2 protein, leading to stomatal closure to conserve water. However, mutations at the OST2 locus can disrupt this response, resulting in continuous stomatal function and potential necrotic lesions.

Using a refined CRISPR-Cas9 framework, researchers were able to achieve a high mutation efficiency (>32%) without causing off-target effects. The resulting ost2-CRISPR mutants demonstrated a significantly enhanced ability to close their stomata compared to wild-type plants, which reduced water loss during drought conditions. This indicates that CRISPR-Cas9-mediated modifications at the OST2 locus can improve drought tolerance by optimizing stomatal responses, thereby enhancing the plant's ability to survive in water-limited environments.



CRISPR–Cas9 alleviates drought stress and promotes plant growth and development.

FUTURE PERSPECTIVES

The future of CRISPR-Cas9 technology in agriculture is highly promising, with the potential to revolutionize food production and tackle pressing global issues. Here's an overview of key future directions

1. **Boosting Crop Yield and Quality**

   Stress tolerance enhancement : CRISPR/Cas9 can be applied to create plants that better withstand environmental challenges like drought, soil salinity, and temperature extremes, helping them thrive in changing climates.

   Improved nutritional content : By modifying genes linked to nutrient production, CRISPR can enhance the vitamin, mineral, and amino acid profiles of crops, making them more nutritious, which can be especially beneficial in addressing malnutrition in vulnerable regions.

   Disease resistance development : Targeting genes associated with vulnerability to pathogens, CRISPR can produce crops with stronger defenses against viruses, fungi, and bacteria, reducing reliance on chemical pesticides and promoting sustainable farming.

2. **Precision in Breeding Programs**

   Faster breeding cycles : Traditional plant breeding methods are time-consuming, but CRISPR/Cas9 enables precise genetic changes, accelerating the development of new crop varieties with desired traits like pest resistance or higher yield.

   Simultaneous trait integration : CRISPR allows for the integration of multiple beneficial traits in plants at once, a process that is much more complex using conventional breeding methods.

3. **Creation of Climate-Adaptable Crops**

   As climate change leads to more frequent extreme weather events, CRISPR can be utilized to engineer plants that are better adapted to these conditions. Editing genes responsible for water conservation, temperature tolerance, or improved photosynthesis can help crops flourish in harsher climates.

4. **Promoting Sustainable Agriculture**

   Reducing agrochemical use : By developing crops that are naturally resistant to pests and diseases, CRISPR can reduce the need for chemical pesticides and herbicides, lowering their environmental impact and contributing to more eco-friendly farming.

   Enhancing nitrogen efficiency : Modifying genes involved in nitrogen uptake can result in crops that require less nitrogen fertilizer, thus minimizing nitrogen runoff and its negative environmental effects.

5. **Precision Agriculture and Tailored Solutions**

   Region-specific crop varieties : CRISPR/Cas9 can be employed to fine-tune crops for optimal growth in specific environments. This allows for regionally adapted plants that better meet local agricultural demands while reducing the need for excessive inputs like water and fertilizers

CONCLUSION

CRISPR-Cas9 technology marks a significant breakthrough in agricultural science, providing the capability to improve crop characteristics, bolster resilience against climate change, and foster sustainable farming methods. This cutting-edge tool facilitates precise genetic alterations, allowing it to tackle essential issues like food security, resource management, and environmental sustainability. As researchers and farmers utilize CRISPR-Cas9 responsibly, it sets the stage for a more efficient, productive, and equitable agricultural landscape, ultimately benefiting consumers.

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ROLE OF DIETARY SOFT ELECTROPHILE SIGNALLING IN THE RESOLUTION OF NEUROINFLAMMATION AND THE APPLICATION OF MASS SPECTROSCOPIC TECHNIQUES TO CHARACTERIZE THE ELECTROPHILIC CHANGES in vivo.

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The leading risk factor for the development and progression of many diseases, including cancer, cardiovascular, and neurological conditions, is aging. Low-grade, sterile inflammation, often known as inflammaging, is a hallmark of human aging. Numerous aging characteristics, such as modifications in energy generation, oxidative balance, or brain function, are linked to and predictive of chronic inflammation (Kempuraj *et al.* 2020). Increased inflammation hastens aging and is a major contributor to death in the elderly. There aren't any recognized preventive measures or interventions available right now that can reduce inflammatory aging and increase longevity. According to epidemiological research, altering one's lifestyle by engaging in regular exercise or adopting a certain food regimen lowers the risk of contracting diseases linked to inflammation. As dietary supplements, numerous botanicals comprising different plant extracts are presently offered with claims of enhancing resistance to age- and inflammation-related neurological alterations (Shen *et al.* 2015). There is also scientific proof that regular consumption of flavonoids and omega-3 fatty acids improves cognitive function in both human and animal models and helps prevent the onset of age-related neurological disorders and increases life span. The underlying molecular mechanism of action of these formulations is still unclear, and these assertions have not been proven. From the following studies that are summarized below, I have tried to highlight some of the major findings that explain the above-mentioned mechanisms in detail and thus link possible answers to the research questions discussed alongwith.

Introduction and Background

Polyphenols are considered as important dietary plant constituents. They are highly studied natural compounds with a wide spectrum of biological activities. But their lack of a specific target or biological function makes them some minor compounds with dynamic residual complexity. They are also considered as pleiotropic in nature (Seigler *et al.* 2021). They have passive roles to play in the specific 'disease-symptom-cure' concept of modern health practise but have significant physiological effects as has been proved in the literature (Maitra et al. 2022). It was in the 1930s when Szent Gyorgyi observed that the crystalline fraction of citrin along with Vitamin C was responsible for the vascular permeability in treatment of scurvy. This led to the concept that plant secondary metabolites aided the vitamins in their function, and that Vitamin C alone was less efficient in treating scurvy. With this hypothesis, more secondary metabolites, especially flavonoids like catechin, quercetin, coumarin were started to being worked upon, in search of a similar "Vitamin P-like activity". And linking these concepts, Seigler *et al.* in a recent review discusses how certain polyphenols can have vital functions even if they are redundant as drug leads. Their mechanism of action is somehow poorly understood. Epidemiological data suggests that a diet rich in colored fruits, vegetables and herbs containing polyphenols, especially flavonoids can lower the risk of chronic, age-associated and neurological disorders (Agarwal et al. 2018). But flavonoids lack essential characteristics of being determined as drug leads. They do not have well defined targets, stability and bioavailability, thus termed as Improbable Metabolic Panaceas (IMPs). And it is due the same reason that the entire class of polyphenols are excluded at early stages of drug discovery pipelines. They give false-positive results in High throughput screening assays and are classified as PAINS (Pan-Assay Interference Compounds). But chemically flavonoids and their metabolites have the same action as electrophilic molecules. They contain essential structural elements that allow them to behave like soft electrophiles, especially the α,β-unsaturated carbonyl groups, due to which they can undergo Michael addition reactions with nucleophiles, mostly the cysteine thiols (Long et al. 2022). This allows them to mimic the physiological role of the lipid-derived essential pro-resolving molecules including lipoxins, marsesins and resolvins, that aid in resolution of neuro-inflammation. It is fairly possible that these flavonoids in combination with omega-3-derived essential fatty acids can act as "essential soft electrophilic molecules" that might resolve neuro-inflammation (Maitra *et al.* 2022). It is further hypothesized that prenylated flavonoids have the potential to cross the blood-brain barrier and impact the Nrf2 pathway, ultimately leading to inhibition of NF-κB signalling and effective protection against neuroinflammation in humans. The effect of Nrf2's anti-inflammatory responses in combination with the decreased production of NF-κB's pro-inflammatory genes as a result of that can pave out new ways for future research in the field. Compounds such as the wide class of polyphenols, that phenotypically demonstrate neuroprotection have been shown to target these pathways (Maitra et al. 2022; Seigler *et al.* 2021). But the concrete mechanism of how these unique compounds actually inhibit neuroinflammation has very poor understanding till date, and there is no established assay that can trace and determine their bioactivity presently as well (Long et al. 2022). It is here that we need to focus our further research on. Some of the widely studied polyphenols have been demonstrated structurally by various scientists who proved their anti-inflammatory and antioxidant activity, which has been plausibly summarized by Seigler et al., as shown in the figure below.

Endogenous electrophilic signaling is being studied thoroughly nowadays in relation to anti-inflammatory immune response pathways like the NF-□B pathway. The ***Kelch*** domains of human Keap1 have been found to have increased sensitivity to the influx of soft electrophiles that are intricately associated with the management of inflammation in aging humans (Lawrence et al. 2001). The wide class of dietary polyphenols, having similar structures as that of soft electrophiles have been shown to have anti-inflammatory immune responses. Despite of being proved as potential antioxidants, it is almost impossible to consider the class of polyphenols as drugs leads because of their lack of specificity. But modern health practice suggests that regular dietary intake of polyphenols in general increases cognitive capacity in humans and delays age-related neurodegenerative disorders, very similar to the activity shown by Omega-3-fatty acids (Tamtaji *et al.* 2019). However, we do not fully understand the mechanism behind this. As we already know, biochemistry is nothing but the movement of electrons across the pool of cells we have.

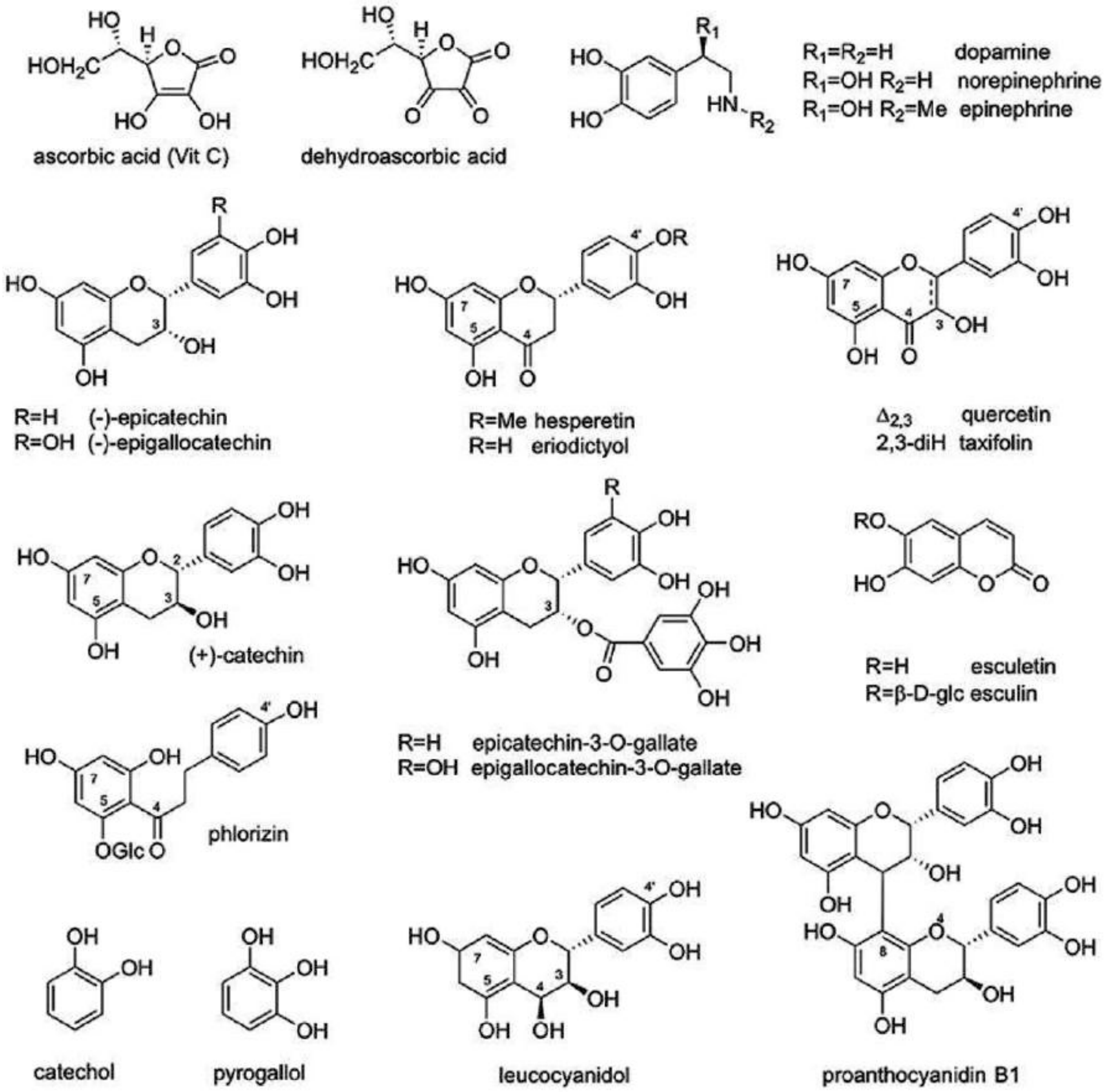


Figure 1: Some of the major polyphenols which have been shown to possess antioxidant and anti-inflammatory properties. (Seigler et al. 2021)

The pool is only replenished by a regular supply of essential electrophiles through our diet. And without these, there will be no one to mediate the movement of electrons, to accept them and transport to a different location. As a simplified version of the complex procedure that revolves across our lifespan, this influx of electrophiles and their sensing by specific protein domains, is what progresses the mechanism of our aging. The increase of inflammation, immune responses, autophagy, and changes in cognitive behavior, all are aspects of these interactions as we age, collectively now termed as a process called "inflammaaging". But the extent of these interactions may be fairly dependent on how regularly our supply of those "essential electrophiles" are fulfilled. And an absolute answer to that question is all those news snippets that we come across every day, that *"it is never too late to add color to your plate."* That how these soft electrophilic molecules are sensed by the proteins involved in the cellular signaling NF-κB pathway, how they upregulate the production of pro-resolving lipid mediators in the presence of certain omega-3-derived precursors, and how they are covalently modified to prevent age-related inflammatory responses. The wide class of polyphenols present in almost all colored fruits and vegetables, contain essential structural elements that allow them to behave like ***soft electrophiles***, especially the α,β-unsaturated carbonyl groups, due to which they can undergo Michael addition reactions with nucleophiles, mostly the cysteine thiols (Liu *et al.* 2015, Sauerland *et al.* 2021). This allows them to mimic the physiological role of the lipid-derived essential pro-resolving molecules including lipoxins, maresins and resolvins, that aid in resolution of neuro-inflammation. It is fairly possible that these flavonoids in combination with omega-3-derived essential fatty acids can act as "essential soft electrophilic molecules" that might resolve neuro-inflammation. It is further hypothesized that prenylated flavonoids have the potential to cross the blood-brain barrier and impact the Nrf2 translocation, ultimately leading to inhibition of NF-κB signaling and effective protection against neuroinflammation in humans. Compounds such as the wide class of polyphenols, that phenotypically demonstrate neuroprotection have been shown to target these pathways. Sulphoraphane has been shown as a competent electrophile which tunes the protein domains for adduct formation (Ahn *et al.* 2010). But the concrete mechanism of how these unique compounds actually inhibit neuroinflammation has very poor understanding till date, and there is no established assay that can trace and determine their bioactivity presently as well. It is here that we need to focus our further research on.

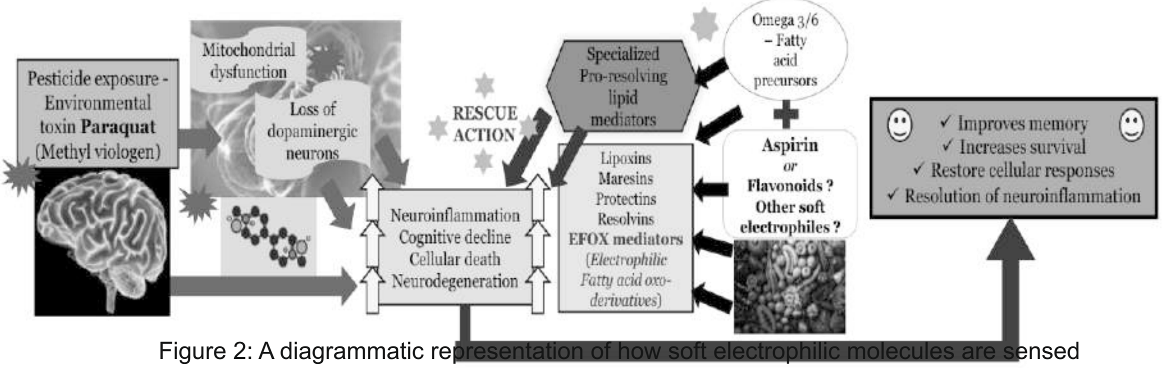


Figure 2: A diagrammatic representation of how soft electrophilic molecules are sensed by the proteins involved in the cellular signaling NF-κB pathway and upregulate the production of pro-resolving lipid mediators in the presence of certain omega-3-derived precursors.

An essential transcription factor for the control of genes involved in cellular responses to oxidative stress and inflammation is nuclear factor E2-related factor 2 (Nrf2). It is considered to be a crucial regulatory route for neurodegenerative illnesses as a result. For instance, there is strong evidence that oxidative stress and inflammation play a substantial role in the development of Parkinson's disease and Alzheimer's disease. Nrf2 is bound by its repressor, Keap1, in the cytoplasm before activation (Colombo *et al.* 2020). The Neh2 domain of Nrf2 is where the Keap1 regulator is connected. However, as Nrf2 is a transcription factor, its activity depends on its presence in the nucleus. Therefore, Nrf2 can translocate into the nucleus and start acting as a transcription factor once the Keap1 regulator is released from the Neh2 domain. Reactive oxygen species (ROS) generate inflammation and oxidative stress, which must be counteracted by this procedure. It is here when specific electrophiles, also termed as reactive electrophilic species (RES) that form stable adducts with the cysteine thiols in the Keap1 Kelch domain to combat the increase of oxidative stress, by mimicking the structure of ROS, but not so identical to them (Long *et al.* 2022) These electrophilic modifications have shown to be covalent and irreversible, thereby conferring manifestating oxidative stress management and conferring neuroprotection.

The Nrf2-Keap1 regulatory pathway is commonly thought to be an important regulator of the antioxidant capacity of an organism. Nuclear factor E2-related factor 2 (Nrf2) is an important transcription factor for regulation of genes involved in cellular responses against oxidative stress and inflammation related to many complex diseases in humans, especially neurodegenerative disorders (Dinkova et al. 2002). Parkinson's Disease and Alzheimer's Disease, for example, both have significant evidence to show that oxidative stress and inflammation are important causes of the disease (Sivandzade *et al.* 2008). Under levels of high oxidative stress, the Nrf2 transcription factor can translocate to the nucleus from the cytoplasm and become an active transcription factor in the nucleus. The responses produced following translocation of Nrf2 involve production of anti-inflammatory cytokines, superoxide dismutases, oxidoreductases, and other enzymes capable of scavenging free radicals. Thus, the activation of Nrf2 becomes a powerful and important mechanism for protecting against oxidative stress and could be a potential target for preventing the negative impacts of these neurodegenerative diseases, or for treating the effects in affected individuals (Sivandzade *et al.*, Helga *et al.* 2008).

Prior to activation, Nrf2 exists in the cytoplasm, and is bound by its repressor, Keap1. Being a transcription factor, it needs to be in the nucleus to be fully functional and active. The Keap1 regulator is bound to the Neh2 domain of Nrf2. So, once the Keap1 regulator is removed from the Neh2 domain, Nrf2 is then able to translocate into the nucleus and begin its activity as a transcription factor (Dinkova *et al.* 2017). This process is essential for counteracting the oxidative stress and inflammation caused by reactive oxygen species (ROS). These reactive oxygen species — peroxides, superoxides, hydroxyl radicals, etc. — are produced during oxygen metabolism in aerobic organisms. Unfortunately, enhanced levels of ROS without the proper means for defense against them, is a direct cause of oxidative stress. The Nrf2 repressor is known as Keap1, and it binds to Nrf2 in the cytoplasm. Upon oxidation or phosphorylation of this Nrf2/Keap1 complex, Keap1 releases Nrf2. Unbound by repressors, Nrf2 is a relatively stable transcription factor, and can translocate to the nucleus (Sivandzade *et al.* 2018).

The binding mechanism of Nrf2 to the antioxidant response element (ARE) is not fully known, but it is known that this ARE sequence in the promoter region of the Nrf2 target genes is bound by Nrf2

Following this binding, the antioxidant response element can initiate transcription of numerous genes useful for counteracting oxidative stress. In a similar context, NF-κB is known to regulate several inflammatory and stress response pathways (Han et al. 2021). These signalling pathways are largely important in the development of age-related diseases. Recent studies have suggested a link between the Nrf2 and NF-κB pathways in the regulation of oxidative stress that is, the interplay between these two transcription factors is a promising target for exploration of oxidative stress relief (Helga *et al.* 2008).

Inhibition of NF-κB Pathway in resolution of neuroinflammation

NF-κB is a transcription factor that controls the expression of numerous genes involved in immune and inflammatory responses. It acts as a molecular switch, turning on the transcription of pro-inflammatory cytokines, chemokines, adhesion molecules, and enzymes that are crucial for recruiting immune cells and promoting inflammation (Hu et al. 2012). While the NF-κB pathway is essential for the proper functioning of the immune system, dysregulation of this pathway can lead to chronic and excessive neuroinflammation, contributing to the pathogenesis of various neurological disorders such as Alzheimer's disease, Parkinson's disease, multiple sclerosis, and stroke (Kelly et al. 2007). Understanding the NF-κB pathway's role in neuroinflammation is not only vital for unraveling the underlying mechanisms of these debilitating conditions but also holds promise for the development of potential therapeutic interventions (Higdon *et al.* 2012). Researchers are actively investigating ways to modulate the NF-κB pathway to mitigate neuroinflammatory responses and restore CNS homeostasis. By targeting specific components of this pathway, it may be possible to dampen excessive inflammation and protect neuronal integrity, offering new avenues for treating neuroinflammatory diseases (Peter et al. 2003). Studies regarding the inhibition of the NF-κB are widespread. There is information regarding the activation process, the downstream genes, and the effects of increased or decreased regulation of each factor. NF-κB, on the other hand, is also activated after its inhibitor is phosphorylated by IκB kinase (IKK), allowing it to translocate to the nucleus. Once in the nucleus, NF-κB has been shown to regulate the expression of several cytokines, such as TNFα, IL-1α, and IL-1β. These three cytokines, as well as many other genes that are upregulated as a result of NF-κB translocation, are pro-inflammatory (Jobbagy *et al.* 2019). As a result, they would be expected to increase inflammation in the brain, thereby increasing the likelihood of developing a neurodegenerative disease. Therefore NF-κB signalling mechanism is also important to consider in researching therapies for such diseases, as the effects of its activation act upon similar genes as the Nrf2 pathway, but for the opposite purpose. It is also hypothesized that Nrf2 activity is hindered somewhere downstream by the increased inflammation in cells (Dinkova *et al.* 2017). NF-κB, on the other hand, must be inhibited to prevent cellular inflammation. Without translocating, NF-κB cannot upregulate all the pro-inflammatory cytokines that it normally does. This is the basis for assertions that Nrf2 and NF-κB are promising targets for prevention of neuroinflammation and neurodegeneration due to oxidative stress (Colombo *et al.* 2020). The figure shown below is a visualization of these processes described above :

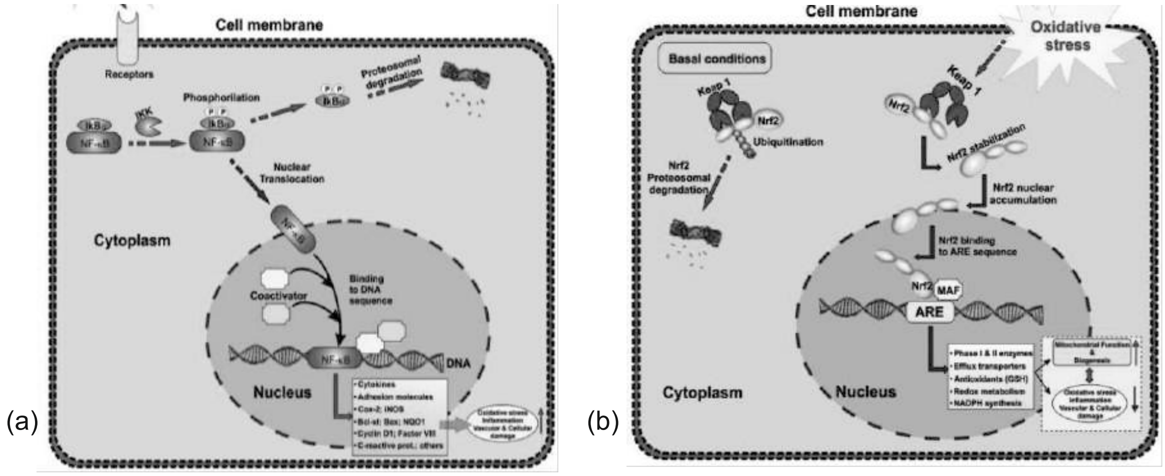


Figure 3a, 3b: The NF-κB cellular signalling pathway activation
under oxidative stress response, as is demonstrated by Sivandzade et al. 2018.

Oxidative stress induced damage in neurodegeneration

When ROS (Reactive Oxygen Species) dominate over antioxidant capacity, oxidative damage like loss of protein function, modification of purines and pyrimidines, a decline in mitochondrial function, and lipid peroxidation is observed (Helga *et al.* 2008, Johnson *et al.* 2008). The Keap1-Nrf2-ARE pathway, however, is partially responsible for maintaining this balance between oxidative and reductive species. Keap1 represses Nrf2 in the cytoplasm by binding to its Neh2 domain through some sort of cysteine interaction. Under conditions of oxidative stress, Nrf2 is freed from the repressive Keap1, and can translocate into the nucleus (Patel *et al.* 2011). Once in the nucleus, Nrf2 induces the expression of a whole host of antioxidant enzymes. With neurodegenerative diseases, often associated with aging, the hypothesis is that biological aging results in an increase in ROS production as well as a decrease in ability to defend against ROS (Lawrence *et al.* 2001). There have been many studies linking mitochondrial toxins to Parkinson's Disease (Paraquat in the mouse model, for example). Administration of drugs that attempt to treat PD have been reported to stimulate Nrf2 activity as part of the cytoprotective mechanism, but more research is needed to thoroughly understand the mechanisms of action (Tran *et al.* 2017).

Studies point out that dopaminergic cell death is the ultimate cause of PD, and that oxidative stressors begin the cascade of events that leads to the degeneration of this important cell type. While it is known that proteasomal function is impaired in PD patients, the effect is the result of a cascade of events, so the specific cause cannot be determined (Greiner *et al.* 2005). The result of this proteasomal impairment, however, is generation of free radicals. Thus, oxidative stress is yet again suggested to be a leading cause of Parkinson's Disease and other neurodegenerative diseases (Kempuraj *et al.* 2020).

Following the cue from oxidative stress induction, specific downstream genes have also been considered in previous studies that are regulated as a result of Nrf2 translocation following its release from Keap1. Specifically, there is considerable downregulation of the TNF-a, IL-1, IL-6, IL-8, MCP-1 genes in various cell lines. All these cytokines are proinflammatory (Song et al. 2006, Gerondakis *et al.* 2014). By inhibiting their activity, one would expect decreased inflammation in a particular cell line, and ultimately, protection against the neurodegenerative diseases that result. Many studies have detailed these effects, through quantitative PCR analysis of pro-inflammatory cytokines TNF-a, IL-1, IL-6, IL-8, MCP-1, and others. With the upregulation of these genes being linked to different neurodegenerative diseases, it seems clear that any pathway able to regulate their expression could be a potential target for therapy (You et al. 2008). And it is here that the soft electrophilic nature of plant flavonoids has a great role to play, in regulation of a pathway such as NF- κB signalling and controlling the expression of those cytokines (Patel *et al.* 2011).

Role of flavonoids in regulation of oxidative stress

Johnson *et al.* (2008) for the first time showed that food derivative curcumin has inhibitory effects on the cytokine-mediated NF-κB pathway by blocking a signal upstream of NF-κB-inducing kinase and IKK. This was proved in using cells of rat and human origin. But this study failed to link a connection to establish the specificity of curcumin in the inhibition process. Following that, many studies thereafter has been undertaken to prove the antioxidant and anti-inflammatory properties of such compounds. Studies by Jobin *et al.* (1999) and Zhang *et.al.* (2001) found that Avicins, a family of triterpenoid saponins from *Acacia victoriae* also contribute to inactivation of TNK-induced NF-κB pathway. Treatement of mammalian cell lines with Avicin G resulted in decreased expression of NF-κB regulated proteins such as cyclooxygenase (COX-2). One of the important highlights of this study was Avicin G did not inhibit the degradation of IκB, but inhibits the nuclear accumulation of the p65 subunit of NF-κB. This clearly suggests that there is a helper or a synergestic action of flavonoids with other compounds that is causing the entire cascade to occur in conjunction.

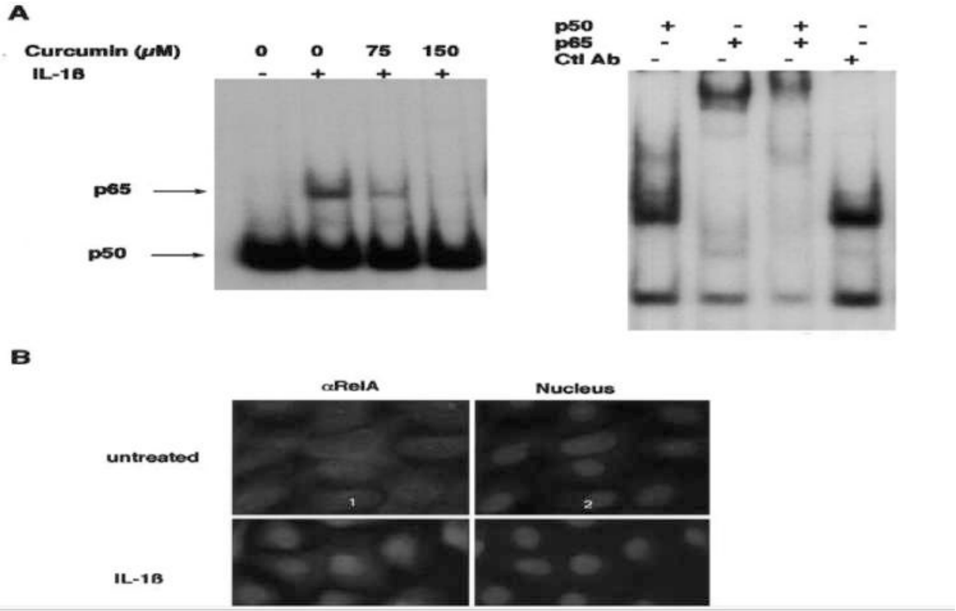


Figure 4: Action of Curcumin on cytokine-mediated NF-κB pathway. (Jobin *et al.* 1999)

Further studies over the decade showed that dietary flavonoids increase plasma very long-chain fatty acids in rats (Toufektsian *et al.* 2008) but failed to clearly study the mechanism or develop an assay to do so. It was somehow just mentioned in one review study that flavonoids might be the key switches that control the long-chain fatty acids to produce transcription factors regulating the NF- κB signalling pathway in neuroprotection with age (Kisseleva *et al.* 2006). But no data has till date proved its validity in in vivo model organisms. Quite similar studies made with polyphenols traced out some of equivalent results regarding the cellular signalling pathways. Anti-inflammatory activity of catechin in mouse macrophages showed that catechin treatement inhibited Nitric oxide production through the downregulation of inducible nitric oxide synthase expression in lipopolysaccharide stimulated ImKCs (Kim *et al.* 2021). Hydrophobic fractions containing polyphenols from wheat inhibited NF-κB and phosphorylation of MAPK proving them to be anti-inflammatory (Scott et al. 2008). Also, some polysaccharides were also shown to attenuate MPP+ -induced injury in PD by regulating NF-κB pathway in mice and cellular models (Guo et al. 2021). But all of them failed to indicate the specificity of the mechanism and establish a proper dosage response by which these compounds were acting on the cellular signalling pathways.

A study by Phi-long Tran *et al.* (2020) details the anti-inflammatory effects of 10-oxomornigrol F. This compound, which is a prenylated flavonoid, is shown to increase ROS presence in the cell, effectively activating the Nrf2 pathway to decrease inflammation. The study not only shows that prenylated flavonoids as soft electrophiles are capable of releasing Nrf2 from Keap1, but it also demonstrates yet another association between Nrf2 and neuroinflammation/neuroprotection. Another important insight onto the possible mechanisms of flavonoids acting as neuroprotective agents was shown by Maitra *et al.* (2021) where Gardenin A and B were studied for their neuroinflammatory responses in Drosophila models. It was shown that Gardenin A and not Gardenin B was able to provide neuroprotection regulating the same NF-κB pathway against environmental toxin of a Parkinsonian phenotype.

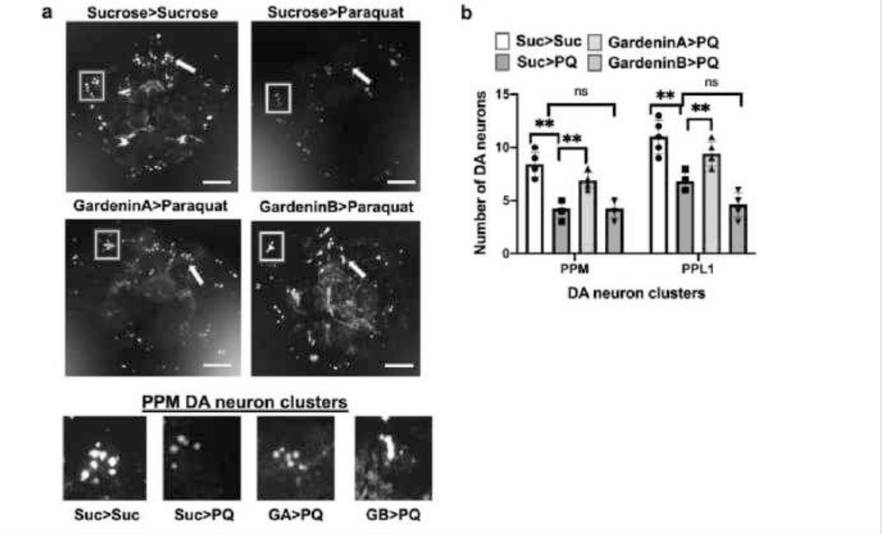


Figure 5: Action of Gardenin A and Gardenin B on a Drosophila model of Parkinson's disease (Maitra et al. 2021)

Available literature has shown that aspirin promotes the resolution of acute inflammation in many ways. Aspirin reduces levels of cyclooxygenase-mediated pro-inflammatory prostaglandins (Grilli *et al.* 1996). They upregulate the production of pro-resolving lipid mediators and triggers the production of anti-inflammatory mono-oxygenated lipid mediators (EFOX). *In-vivo* studies have shown that they regulate the ectopic Toll/NFkB and JAK/STAT signalling pathways. The production of such electrophilic pro-resolving lipid mediators (EFOX) was detected in *Drosophila melanogaster* models when the flies were treated with aspirin. A study conducted by Panettieri et. al. has proved that the production of 13-HODE (Hydroxyoctadecadeioic acid) and its oxidized form 13-EFOX-L2 lipid mediators are triggered in model organism Drosophila melanogaster flies when the larvae are treated with aspirin (Panettieri *et al.* 2020). Aspirin is acetylsalicylic acid commonly derived from the bark of willow tree (*Salix alba*). Flavonoids have similar physio-chemical properties to that of aspirin which makes function in a theoretically similar form as well (Carmo *et al.* 2021). So, it is hypothesized that mammalian models when prefed with flavonoids may show a change in concentration of the lipid mediators. But, to first trace and detect the presence of these lipid mediators in the mammalian models, precursors of the lipid mediators like linoleic acid and α-linoleic acid are required. These precursors will help in determining the actual concentration of the lipid mediators and their substantial quantification through lipidomics.

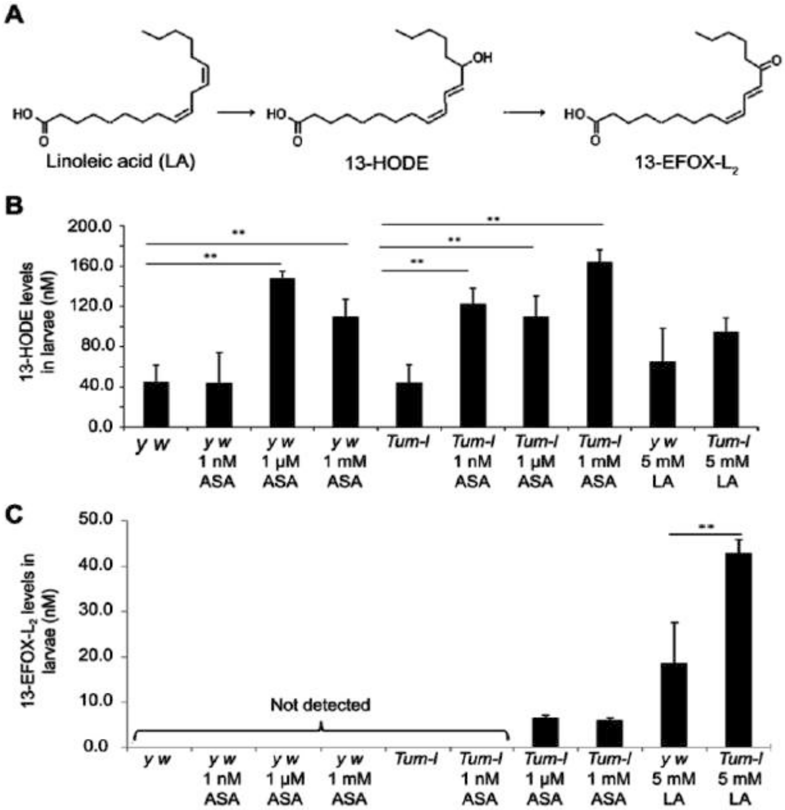
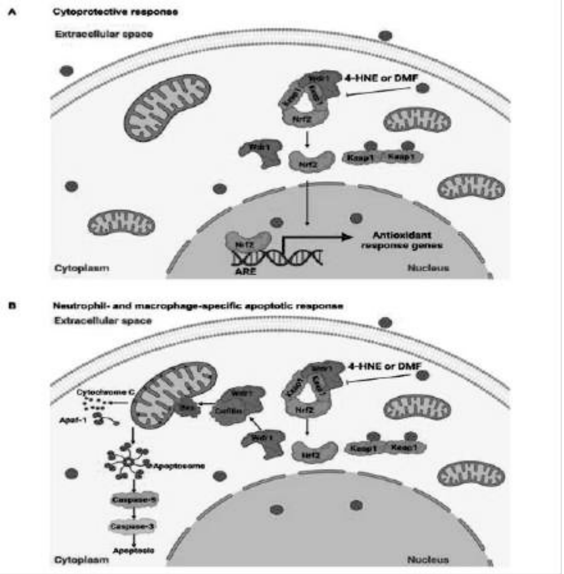


Figure 6: A study by Panettieri et al. demonstrated that aspirin triggered production of 13-HODE lipid pro-resolving molecule in Drosophila melanogaster blood tumor metainflammation models.

Endogenous electrophiles as signaling molecules.

Two studies thoroughly discussed some recent findings from their work that highlight the aspect on how reactive signals function on specific protein targets and then modulate that protein's properties to propagate signals. So far, we have seen numerous evidence about the presence and activities of many such pleiotropic endogenous electrophilic species present in our system, but we are unsure of their source and mechanism of action. However, this study vividly presents how signaling alterations can take place even in smaller subcellular locations with low ligand occupancy and where the target protein domains are not found to have their conventional activity. The data also shows that fascinating signaling mechanisms with unexpected pathophysiological effects might happen when an electrophile hits the target. And for our simplification we might consider that the electrophile here is able to interact with a particular target protein in the right precise cellular environment.

They used the T-REX (Toolbox for Reactive electrophilic species) method which is a method to selectively target a specific protein with a given electrophile of choice in a cellular system. Their source of electrophile was the Tecfidera drug approved against multiple sclerosis which includes functions like suppression of immune response and resolution of inflammation. They studied the electrophile association with human Keap1 domain in multiple cellular compartments, involved in the process of caspase3 and caspase9 activation signaling mechanisms. Their findings were the first to emphasize an electrophile binding protein acting as a long-distance signal. They highlight a synergistic effect through their studies between how proteins must be precisely tuned to react with the electrophile and also undergo conformational changes in order to allow the electrophile to communicate. This study indeed throws light on uncovering new modes of signaling actions, cellular associations and disease progression which broadens our understanding of definite protein-electrophile associations as future therapeutic targets.



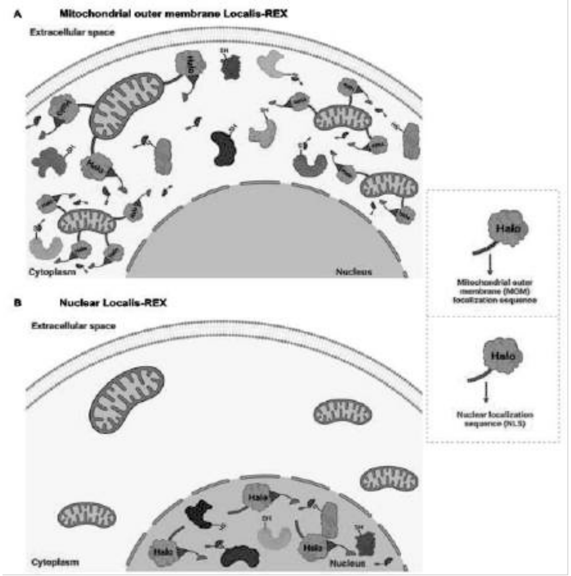


Figure 7A, 7B: Electrophile engagement of human Keap1 with dimethyl fumaarte(DMF) indicating translocation of Nrf2 into the nucleus in presence of antioxidant response genes in a cryoprotective response, as well as the same effect in neutrophils and macrophages. Figure 8A, 8B: Locale-specific electrophilic-signal responsivity index (Localis-REX) technology used to detect electrophilic signaling in organellar proteomes- mitochondria and cytoplasm.

Electrophile-toolbox targeting Keap1 and developing specific quantification methods.

A few studies (Mufson *et al.* 1994, Zhang *et al.* 2016) plausibly summarizes the T-REX approach for small-molecule-signal-specific modification of the Keap1 protein by the redox-signaling actions of various lipid derived electrophiles (LDE). The findings show Keap1 to be a promiscuous electrophilic substitution sensor that may react to distinct electrophilic signals with equivalent efficacy, encouraging Nrf2-ARE activation of similar strength. By blocking Nrf2 degradation, whole-cell electrophile flooding causes ARE to be upregulated. The Nrf2-ARE activation seen after whole-cell electrophile treatment is partly attributed to Keap1, a highly cysteine-rich protein that is well-known *in vitro* for forming covalent adducts with a variety of electrophiles, including LDEs. Keap1 functions as a redox-dependent ARE-regulator through direct interaction with small-molecule electrophiles. A similar approach can be utilized to target specific downstream genes in a *Drosophila* model. This is extremely relevant as it highlights LDEs as major endogenous small molecules regulating redox-dependent intracellular communications and successfully designs a protocol to quantitate their reactivity with Keap1 domains in a cellular setting.

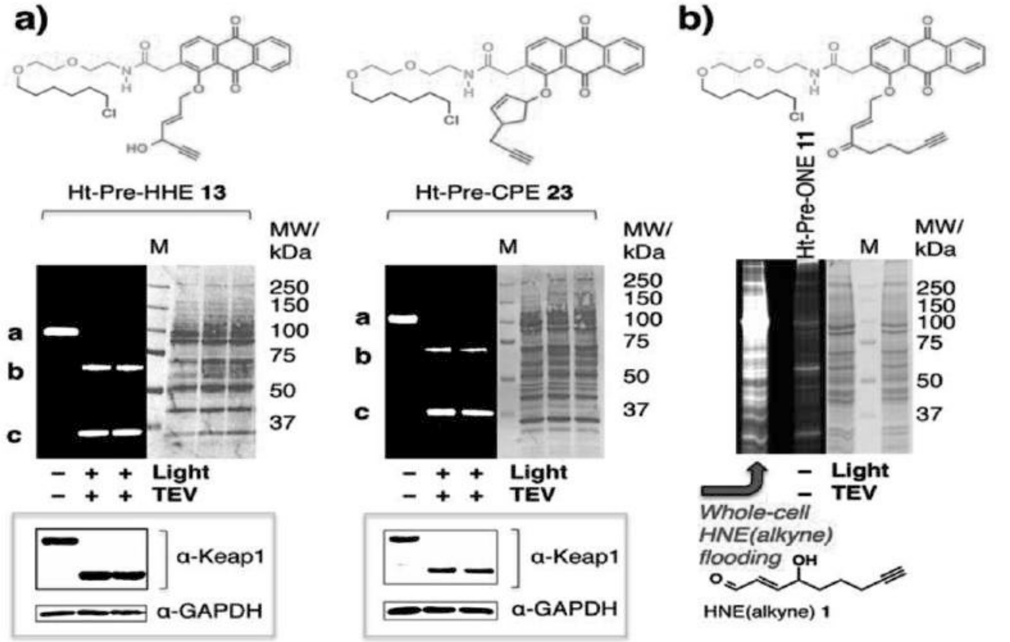


Figure 9(a), (b): Results of T-REX assisted Keap1-specific electrophilic adduction (addition of HNE) compared to whole-cell electrophilic flooding. The results clearly indicate specific cysteine fragments where the electrophilic adducts are formed in the Keap1 domain.

Cell signaling by reactive lipid species.

Previous research highlights the enzymatic and non-enzymatic control of lipid peroxidation and production of lipid derived electrophiles which participate in cellular signaling. This gives an excellent foundation to the research hypothesis, about the fatty acid derived precursors which increase the production of lipid mediators by the influence of dietary soft electrophiles, that also included the class of lipid electrophiles derived from omega3/6-fatty acids. A significant proportion of the oxidized lipid products are electrophilic in nature, the RLS (reactive lipid species), and react with cellular nucleophiles such as the amino acids cysteine, lysine, and histidine (Carmo *et al.* 2021). Cell signaling by electrophiles appears to be limited to the modification of cysteine residues in proteins, whereas non-specific toxic effects involve modification of other nucleophiles. RLS have been found to participate in several physiological pathways including resolution of inflammation, cell death and induction of cellular antioxidants through the modification of specific signaling proteins (Fantin *et al.* 2019). This presents the idea of "electrophilic signaling domains," where the formation of the lipid electrophile occurs near to the location of the signaling protein that contains thiols. Additionally, sccientists suggest that glutathione and its companion enzymes protect the signaling domain from unchecked electrophilic stress, which can be a great clue to determine the dosage optimization in my model organism.

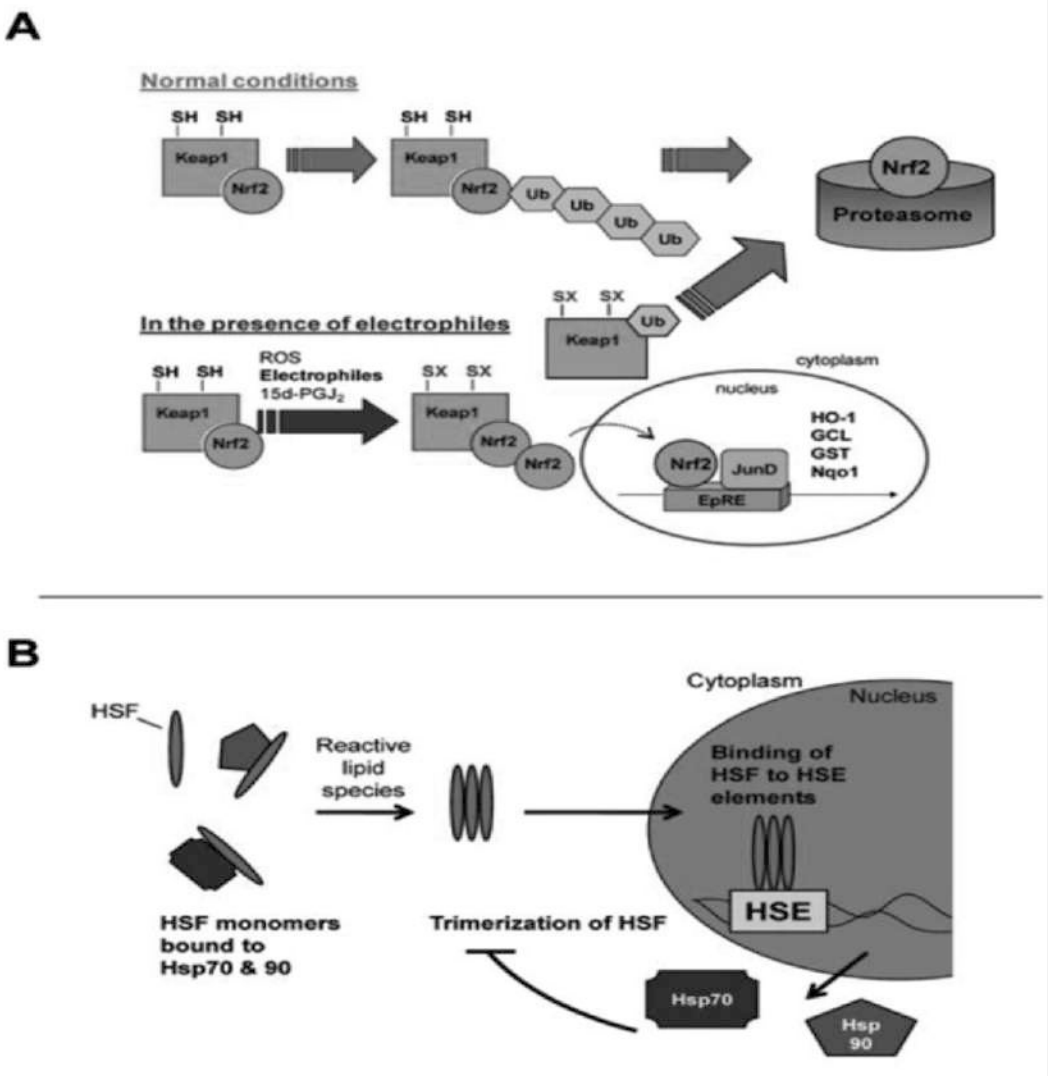


Figure 10A,10B: Modification of protein targets by the action of RLS (Reactive Lipid Species).

Keap1- the cysteine-based mammalian intracellular sensor for electrophiles. The research group led by Dinkova-kostova and colleagues have put forward excellent summarized reviews and have carried out substantial in vivo assays of reactivity assessment and adduct formation of various plant-derived natural products on the electrophile-sensory Kelch domain of Keap1 protein. Molecular data show the involvement of these electrophilic sensory activity of Keap1 in the inhibition of the evolutionarily conserved NF-κB pathway. The transcription factor NF-E2 p45-related factor 2 (Nrf2), which orchestrates a complex transcriptional program in response to environmental challenges brought on by oxidants, electrophiles, and pro-inflammatory agents, is the best-characterized substrate of Keap1 (Ghosh *et al.* 2008). This allows adaptation and survival under stressful circumstances. Reactive cysteine residues in Keap1 serve as sensors for endogenously produced and exogenously encountered small molecules (referred to as inducers), which are distinguished by their reactivity with sulfhydryl groups. Inducers alter Keap1's cysteine sensors, making it less able to recognize and target Nrf2 for ubiquitination and destruction. As a result, Nrf2 builds up, moves into the nucleus, and activates the transcription of its target genes, which produce a vast network of cytoprotective proteins. As characterized by the authors, there are five most reactive cysteine fragments of the Keap1 that can tracked in vitro. The use of HPLC analytical techniques combined with traditional molecular assays developed to characterize these fragments can be a method to quantify the lipid mediators in my research question in vivo as well.

Michael addition reactions of α,β-unsaturated carbonyls to amino acid and protein thiols.

The difference in reactivity of various natural products affects the extent of adduct formation of the α,β-unsaturated carbonyls to amino acid and protein thiols (Ghosh *et al.* 2008). This gives an insight onto the fact that the various classes of compounds from dietary sources that we might use in our research as the test samples might not have the same extent of reactivity to all the cysteine fragments equally. The reactivity between an adduct and a certain Cysteine is affected by a variety of circumstances, however the data show a number of significant characteristics. The first is that different electrophiles vary in their level of reactivity, because of their nature and structural composition (Khan *et al.* 2012). A second key finding is that, for many of the systems investigated, and particularly the low-molecular-mass species, the rate constants fluctuate with the Cysteine pKa value; this appears to be a fair indicator for the species' reactivity with the adduct-forming electrophiles (Lehnardt *et al.* 2003). The accessibility of the Cysteine residue is significant, which is the third main finding.

The effects of omega-3 fatty acids and vitamin E co-supplementation on gene expression related to inflammation. Many studies show that Omega-3 fatty acids and vitamin E co-supplementation for 12 weeks significantly improved TNF-, PPAR-, and LDLR gene expression in Parkinson's disease patients, but had no effect on IL-1 or IL-8 (Jagatha et al. 2008, Javed et al. 2010, Gerondakis *et al.* 2014). A study also looked at how the co-supplementation of omega-3 fatty acids and vitamin E affected the expression of genes associated to inflammation, insulin, and lipids in Parkinson's disease patients (Tamtaji *et al.* 2019). After the 12-week intervention, patients with Parkinson's disease who received co-supplements of omega-3 fatty acids and vitamin E showed decreased gene expression of tumor necrosis factor alpha (TNF-alpha), compared to those who received the placebo. Omega-3 fatty acids and vitamin E co-supplementation also increased PPARgamma (peroxisome proliferator-activated receptor) and decreased oxidized low-density lipoprotein receptor (LDLR) expression in PD patients compared to placebo. This was the first randomized human trial showing the effects of co-supplementation of Vitamin E and omega-3-FA derivative.

Flavonoids regulate lipid droplet biogenesis in Drosophila.

As is already discussed, we have seen many detailed reviews of the possible mechanistic pathways of electrophile signaling and adduct formations in specific protein domains in vitro. We have also seen numerous evidence in model organism systems with regards to the use of natural products as the electrophilic adducts. But we still do not have a properly optimized assay to quantify the reaction products and validate the importance of those covalent modifications. This study is the first of its kind to validate the regulation of lipid droplet biogenesis by the effect of flavonoids, thereby throwing light on the aspect that flavonoids are capable of synthesizing lipid -derived pro-resolving mediators from their respective precursors (Fantin *et al.* 2019). By analyzing the biogenesis of lipid droplets (LDs), the authors were able to use *Drosophila melanogaster* as a model system to look into the effects of a particular set of flavonoids on larval tissues. In our work, fruit flies were raised on diet enriched with xanthohumol, isoquercetin, and genistein, and the tissues from the larvae were examined using an LD marker. After treatments, the total mRNA expression of the two key triacylglyceride-producing enzymes, Minotaur and Midway, was assessed. Among the studied flavonoids, xanthohumol and isoquercetin were found to be effective tissue-specific regulators of LDs biogenesis, causing a decrease in fat storage in fat bodies and an accumulation of LDs in nerves. Our findings support the idea that certain phytochemicals may operate as protective mechanisms against intracellular stress because LDs have been proposed to do so in nonadipocyte cells. This information supports the idea that certain phytochemicals may have potent modulatory effects on the *in vivo* synthesis of Lds.

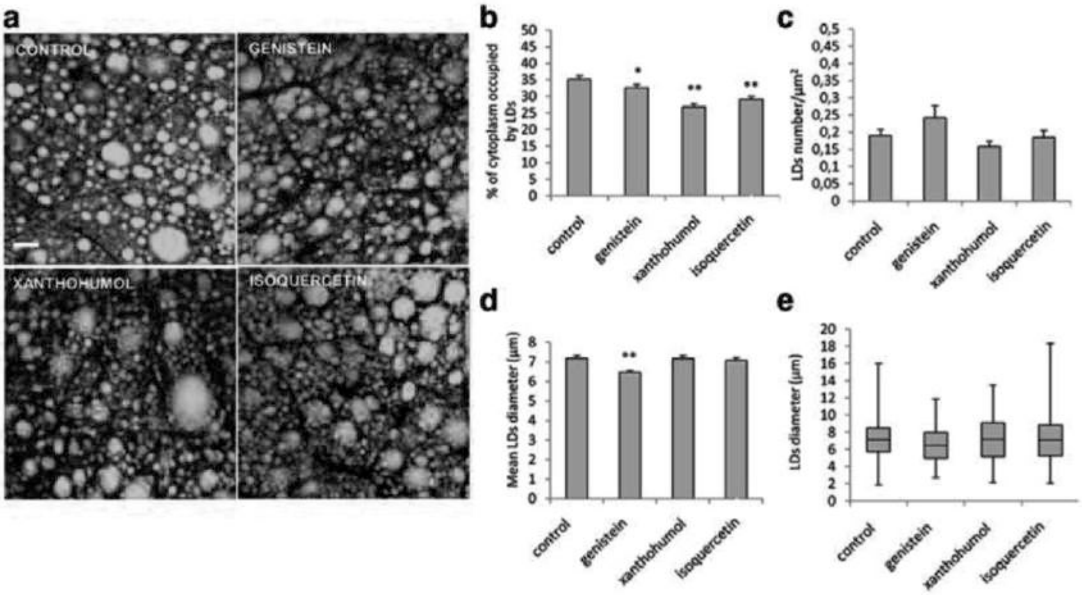


Figure 11: Lipid droplet synthesis in the larval bodies of Drosophila melanogaster under the effect of various plant-derived natural products.

Substituted dietary foods like mushrooms as potential sources of similar soft electrophiles

Zhang *et al.* 2017, characterized certain unique compounds from wild mushrooms *Phellinus baumii* containing the same α,β-unsaturated carbonyl groups to have potential inhibitory effects against tumour-cell proliferations in cell lines such as K562 and MCF-7 active against cancer progression. Following similar assay methods which involve solid state fermentation, Liu *et al.* 2019 identified some novel polyketides from a coral fungus *Penicillium sclerotiorin* which showed anti inflammatory activities by downregulating the expression of COX-2 genes.

Similar polyketides were also isolated from *Aspergillus rugulosa* having anti inflammatory actions by inhibiting the transcription of TNF-α pro inflammatory gene. Hu et al. 2017, also showed that a polyphenol rich extract from common edible mushroom *Pleurotus eryngii* showed anti inflammatory function *in vitro* against the growth of RAW264.7 cells. These RAW cells were also shown to be affected by ethanolic extracts from *Pleurotus giganteus*, the widely edible mushroom via STAT 3 and COX-2 pathways (Baskaran *et al.* 2017). Similarly, polyphenols from extracts of certain bamboo mushrooms have recently been shown to have anti-inflammatory activity in inhibtion of the NF-κB pathway (Han *et al.* 2021). The SH-SY5Y cells were earlier shown to be inhibited by the neuroprotective activity of chrysogenamide A, yet again a soft electrophile, extracted from endophytic fungus *Cistanche deserticola* (Lin *et al.* 2008).

In a function similar to prenylated flavonoids, certain prenylated alkaloids like asperversiamides from marine derived fungal species were structurally elucidated and it was seen that these also posses the α,β-unsaturated carbonyl groups. Their structural data is somewhat similar to the flavonoid baicalein that has been quite recently proven to negatively regulate progesterone target genes in vivo (Li *et al.* 2022) and sophoraflavone M shown to be inhibiting both NF-κB and JNK/AP-1 signaling pathways (Han *et al.* 2021).

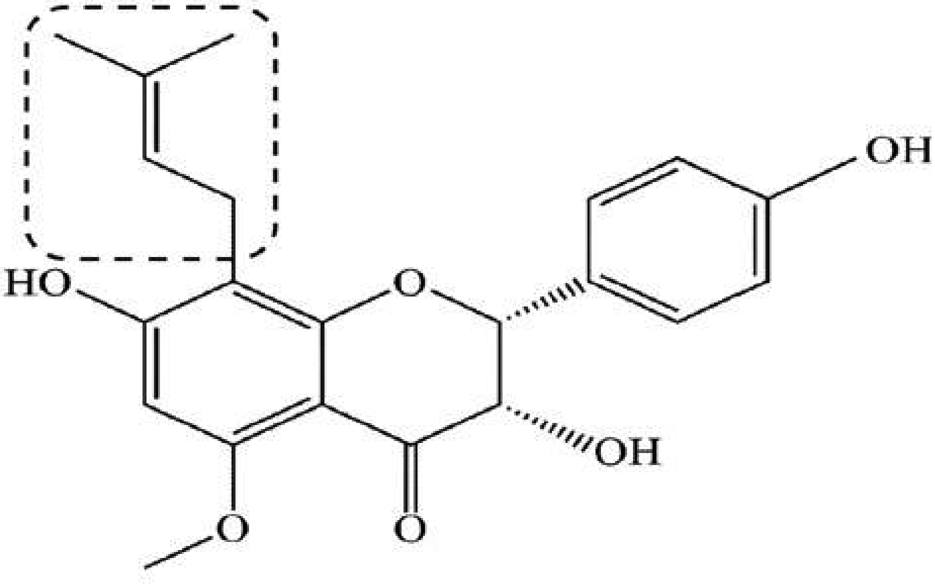


Figure 12: Structure of sophoraflavone M described by Han et al. 2021.

Use of mass spectroscopic techniques to screen electrophile adducts from complex matrices.

The final challenge is to be able to develop a concrete analytical assay which can quantify and characterize these covalent modifications in Keap1, thereby validate the importance of these dietary soft electrophiles in the resolution of neuroinflammation. Two consecutive studies by Kutzner *et al.* (2019) and Jang *et al.* (2021) show that mass spectrometry can release, trap, and identify electrophiles that have irreversibly altered Keap1 as -mercaptoethanol adducts. The first assay was validated using isoliquiritigenin and sulforaphane, two well-known ARE activators that target Keap1. Sulforaphane was injected into a cocoa extract to see if the assay could recognize electrophiles that change Keap1 in complex matrixes. Sulforaphane adducts that had been liberated from Keap1 were then identified using LC-MS/MS with high resolution mass spectrometry and accurate mass measurement. In complicated natural product mixtures that reversibly change Keap1 but cannot be found by MALDI-TOF MS, this screening technique enables the discovery of prospective chemoprevention drugs.

In the second study, an ALS (Amyotrophic Lateral Sclerosis)-induced Drosophila model's brain tissue was examined using matrix-assisted laser desorption/ionization-mass spectrometry imaging (MALDI-MSI) to look into changes in phospholipid distribution. Several hundred micrometers or smaller fly brain tissues were harvested using a fly collar to acquire repeatable tissue sections of comparable sizes. The distribution of phospholipids, such as phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidic acid (PA), phosphatidylserine (PS), and phosphatidylinositol (PI), was significantly different between the control group and the ALS group, according to MSI of brain tissues of Drosophila cultured for 1 or 10 days. Additionally, when the culture duration rose from 1 to 10 days, the lipid profile as determined by phospholipids changed. These findings imply that MALDI-MSI can be used to identify disease markers based on lipid metabolites.

Conclusion and Future directions

Dietary soft electrophiles, even if not drug leads have the pharmacological potential of being evolutionarily optimized drug-like natural compounds. Their bioactivity in neuroprotection has been widely studied. But the mechanism of their action lacks concrete literature and data. My future research is thus, to focus on how they aid the essential pro-resolving lipid mediators, in a way similar to the action of vitamins. Fatty acids were also initially thought to be vitamins with a similar path of action as polyphenols and other soft electrophiles. It was also said the resolution of neuro-inflammation is a self-regulatory process in the human brain. But in the recent years it has been proven that essential lipid-derived fatty acids are the ones that produce certain pro-resolving lipid mediators which confer neuroprotection in humans, as well as post-translationally modify some of the downstream processing genes involved in the neuroinflammation resolving pathways. So, it is fairly possible that flavonoids are those inevitable entities that help the pro-resolving molecules to resolve neuro-inflammation.

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ARTIFICIAL INTELLIGENCE IN CROP BREEDING AND PLANT BIOSINALLING

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Semester-VI

ABSTRACT

Artificial intelligence (AI) have transformed many industries and agriculture to a great extent. In plant breeding AI techniques speeds up the development of new crop varieties with desired features. This article covers the machine learning techniques for trait prediction, genomic selection, phenotype-to-genotype mapping, as well as the state of AI applications in plant breeding. Along with the above mentioned findings fields the article also ways to improve breeding precision and efficiency by integrating AI with other breeding techniques like genome editing and marker-assisted selection.All things considered, AI offers tremendous potential to transform plant breeding by facilitating quicker and more accurate cultivar development, eventually leading to enhanced global food security and sustainability.

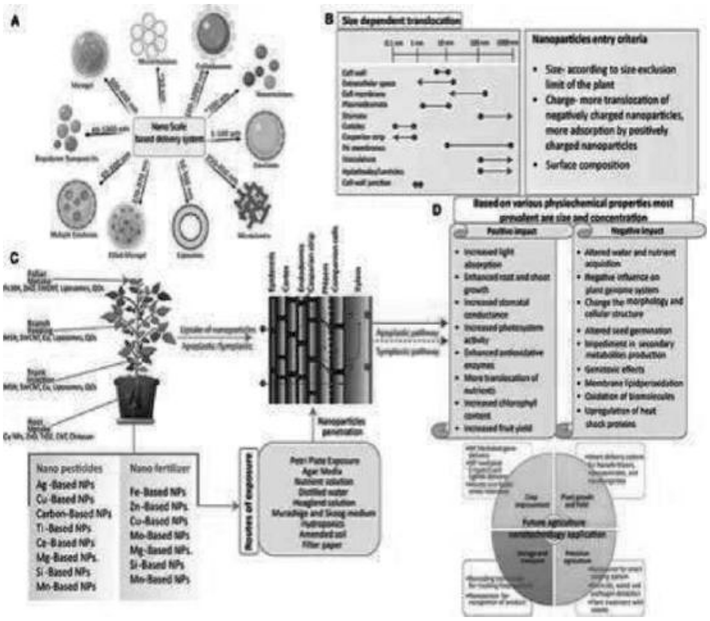
NANOTECHNOLOGY IN PLANT BREEDING

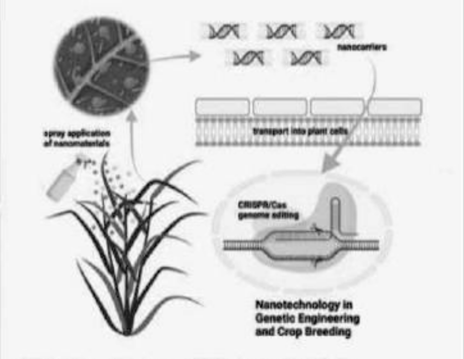
Introduction

Nanotechnology is a prospective field with multiple applications across various areas of modern science, including physics, pharmacology chemistry, computer science, agriculture and engineering [1]. The distinct physical, chemical and biological properties of nanoparticles (NPs) give them the ability to modify typical chemicals and devices [2]. NPs are defined as natural and artificial materials with at least one dimension ranging from 1 nm to 100 nm and these materials can be organic, inorganic or polymeric compounds [3]. With the rapid growth of the population and deterioration of the environment, traditional agriculture is facing unprecedented challenges [3]. Fertilizers and pesticides have played pivotal roles in satisfying food production needs for decades [4]. However, excessive use of these chemicals also hinders sustainable agricultural development [5,6].Increased use of nanotechnology could provide innovative solutions to improve sustainable agriculture, which would also fulfill food demands [7]. Current studies have shown that nanotechnology can be widely used to address various agricultural problems,such as excessive use of fertilizers and pesticides, and plant stress induced by extreme climate [8–12]. Besides, nanomaterials significantly promote plant growth, seed germination and stress tolerance [3]. Moreover, applications of nanotechnology also include plant growth status monitoring, rapid and simple genome modification and transgene expression in intact plant cells [13,14].

NANOTECHNOLOGY IN SEED GERMINATION

Nanotechnology has been used in various aspects of agricultural production, such as seed germination and plant growth, to increase crop yields and quality (Figures 2 and 3).Seed germination is a refined and fundamental biological process associated with environmental factors, genetic traits, and soil parameters. Recently, some studies have shown that NPs such as CNTs, silicon dioxide (SiO_2) NPs, ZnO NPs, titanium dioxide (TiO_2) NPs and even gold (Au) NPs have positive effects on seed germination in crop plants, including tomato, wheat, rice, pearl millet, soybean, barley and maize [12,53–59]. Seed germination is related to antioxidant enzyme activities and the contents or utilization rates of water and oxygen [60]. For example, Au NPs significantly increased the germination rate of pearl millet compared to that of untreated plants [54]. The seed germination rate of wheat treated with ZnO NPs was increased compared with that of the control group [61]. The two NPs mentioned above both have the ability to increase antioxidant enzyme activity. TiO_2 NPs are beneficial to promoting seed germination, and exogenous treatment with TiO_2 NPs enhances the seeds absorption of water and oxygen, leading to decreased germination time. For instance, tomato seeds soaked with TiO_2 NPs exhibited a germination percentage increased by approximately 8% compared with the untreated control [59]. Another study has revealed that TiO_2 NPs stimulate seed germination and dramatically decrease mean germination time in wheatgrass [57]





Schematic diagram of the applications of nanotechnology in genetic engineering and crop breeding. Plasmids containing genes that encode Cas and the sgRNA are delivered into the plant cell through Agrobacterium-mediated transformation, protoplast transfection, particle bombardment, or even spray application with NMs. The CRISPR/Cas genome-editing system consists of the Cas endonuclease, which can site-specifically cleave double-stranded DNA, and an sgRNA that hybridizes to about 20 nucleotides of the target sequence

1. Nanotechnology in seed germination

2. Application of nanotechnology in crop breeding

PLANT BIOSINALLING IN CROP PLANTS

Due to the limited selection of herbicides, the lack of labor, high costs for manual weeding, and the increasing demand for organic foods, the development of automated weed control systems to meet the real-time plant care in the field has received increasing attention.

Hyperspectral imaging differs from non-imaging spectroscopy and color imaging in that it takes numerous narrow-band images from a continuous spectral region, generating the spectra of all pixels in an image . Hyperspectral models using the absorbance in the region of 1700 to 2320 nm achieved over 99% classification rates for discriminating nightshade weed from tomato plant . Based on ground-based hyperspectral imaging (400–1000 nm), Bayesian classifiers and artificial neural network (ANN) were examined to discriminate crops such as lettuce, field pea, canola and tomato from different weeds grown under various sunlight intensities with high accuracies (88% to 94%). However, it takes a lot of time and effort to effectively remove the large amount of redundant information contained in the full wavelength range to simplify the model and improve the speed of on-line detection.

Crop plant signaling is a new robot-plant interaction technique that allows weed/crop differentiation based on photographing of a machine-readable fluorescence signal applied to crop plants rather than the weed. Fluorescent compounds in the plant at very low doses contain fluorophores that can generate very strong fluorescent emissions when excited by specific wavelengths. The fluorescent signals can be then captured by a fluorescence macroscope. According to the attributes of labels, signaling approaches can be divided into three categories : physical markers (such as plant labels), biological markers (such as FPs), and chemical markers (such as systemic crop signaling compounds and topical markers). The following are the examples of their applications for automated identification of the location of target plants.

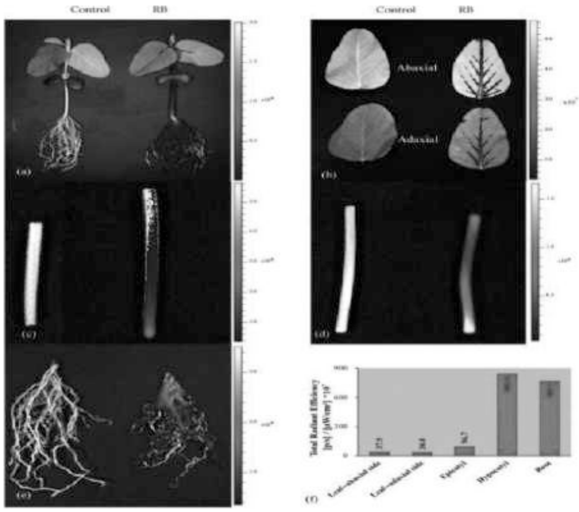
Types of signalling markers

Physical markers: Physical markers refer to the use of inexpensive and biodegradable plant labels made from polylactic acid or maize-based plastic. The plant labels painted with orange, green, or pink fluorescent paints were placed next to seedling stems to provide a unique signal used to localize the crop plants. Because of the fluorescent signal of the plant label under ultraviolet (UV) excitation light, the crop signaling system equipped with a camera was able to detect the occluded and non-occluded crops

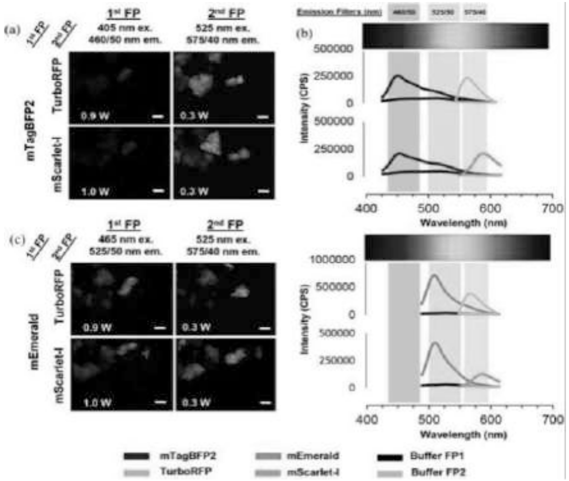
(including tomato and lettuce) with an accuracy of 97.8% at travel speeds up to 3.2 km h−1 in fields with a high density of weeds

Biological markers :

Biological markers are widely used as indicators to measure certain biological conditions. FP is a very efficient genetically encoded biological marker for imaging living cells and tissues (Dixit et al., 2006; Xian et al., 1999). The most common categories are red, green, cyan, yellow, and green-to-red FPs (Chudakov et al., 2010; Hao et al., 2007). Monitoring plants based on fluorescent markers is not a completely new method as it has been proposed for detecting transgenic plants (Stewart Jr, 2005). For instance, the fluorescence of green FP was used to indicate the characterization of protein synthesis in transgenic plants (Richards et al., 2003). The FP produced specific colors was used to screen diseased plants (Wang et al., 2007). Also, FPs expressed in plants can generate a specific signal for classification of crops from weeds. Results showed that the fluorescence generated from green FP under blue (470 nm) excitation light extensively appeared in plant leaves, which helped to identify crops from weeds (Raja et al., 2019). Rigoulot et al. (2019) developed a fluorescence-based platform for imaging of plant canopies that expressed multiple FP genes in leaves.



1. Imaging of Rh bond b(Rb) in soybean seedling



2. Fluroscence measurements in fluroscent coexpressed protein

Chemical markers : Systemic crop signaling compounds are applied to the seedling roots of transplanted crops or through the seed coat to crop seeds that can be sown directly prior to planting, then they are transported systematically to the stem or the foliage of a plant for detection (Su et al., 2020a). Lappartient et al. (1999) found that sulfate can move up into leaves after being absorbed by plant roots. The lipophile property of a compound expressed in terms of the log Kow affects its mode of action and absorption in plants (Salanenka and Taylor, 2006). Molecules with log Kow between 0 and 2 are considered to move more readily in plants (Hsu et al., 1990). The fluorescent tracers mainly originate from chemical families including arylmethane dye, azine dye, coumarins and xanthenes (Wang et al., 2020). Such markers for agricultural use should be cost effective and safe. As an inexpensive xanthene fluorescent compound, the use of Rhodamine B (Rh B) (no more than 60 ppm on the treated seed) does not have a negative impact on the environment or public health based on the statement U.S. Environmental Protection Agency (EPA) (Su, 2020). Studies have shown that Rh B (Log Kow = 1.5) was more easily absorbed by broad bean leaves rather than hydrophilic dyes such as Oregon Green 488.

HOW PLANT SIGNALLING HELPS IN AGRICULTURE

Plant signaling can help in agriculture in many ways, including :

Pest management : Plant volatile-mediated signaling can be used to control pests, weeds, and parasitic weeds through push-pull systems.

Crop yield : Plants respond to environmental changes through long-distance signaling, such as leaf-to-leaf signals that mediate photosynthesis. This information can help optimize crop yield.

Symbiosis : Plants communicate with microorganisms, such as arbuscular mycorrhizal (AM) fungi and Rhizobia, to uptake nutrients like phosphorous and nitrogen.

Defense : Plants exude secondary metabolites that act as defensive signals against pathogens and insects.

Plant identification : Crop plants can be labeled by applying signaling compounds to their seeds, roots, stems, or leaves. This can help distinguish crops from weeds, which could lead to more precise weeding on a commercial scale.

Plant growth : Plant hormones can be used to promote growth, such as in rooting powder that can help stem cuttings develop roots quickly.

AI IN AGRICULTURE: THE INDIAN PERSPECTIVE

India, with its vast agricultural landscape, is poised to benefit immensely from AI adoption in farming. Let's delve into some key figures and developments specific to India :

1. **Agriculture's Dominance**

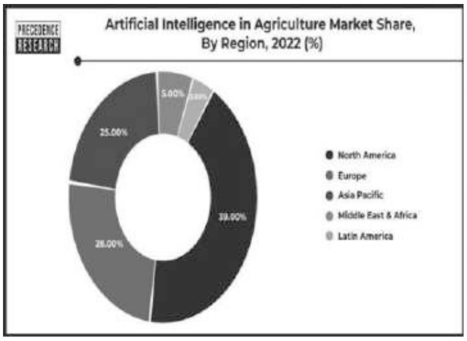
   Agriculture plays a pivotal role in India's economy, contributing over 15% to the country's GDP and employing nearly 50% of its workforce. The adoption of AI in agriculture is crucial to enhance productivity and livelihoods.

2. **Government Initiatives**

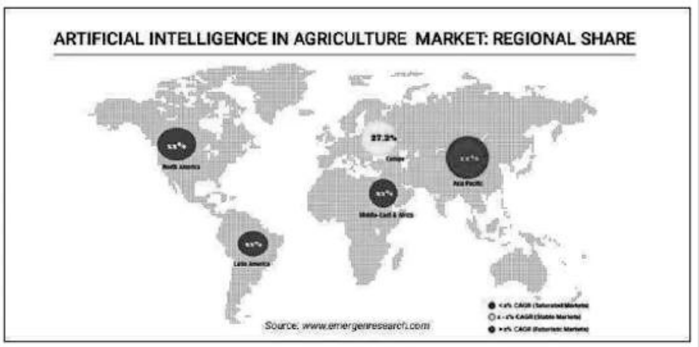
   The Indian government has recognized the potential of AI in agriculture and launched initiatives like the "Krishi Sanyog" program, aimed at promoting AI-driven solutions for farmers. Such initiatives are expected to drive AI adoption in rural India. (Barman *et.al.*, 2023)

3. **Start-up Ecosystem**

   India boasts a thriving agritech start-up ecosystem, with companies like CropIn and AgNext leading the way in AI-powered solutions for farmers. These startups are addressing challenges such as crop disease detection and yield prediction.



1.Pie chart showing AI in agriculture market share



Source: www.emergenresearch.com

2. Map showing AI in agriculture market by regional share

FUTURE PROSPECTS AND CONCLUSION

There has been a notable increase in the discourse surrounding artificial intelligence (AI) in recent times, which has sparked discussions about its potential uses worldwide. Modern plant breeding is required to capitalize on the digital revolution. In order for research and breeding to succeed in the future, they must weigh farmer demands against computergenerated recommendations. Additionally, AI will change how businesses grow and compete globally by bringing new manufacturing concepts that will increase profitability. However, there are drawbacks and issues with using AI in plant breeding, such as issues with data quality, model interpretability, computational resources, and ethical considerations. In order to fully utilize AI in crop breeding while guaranteeing a fair and responsible implementation, these issues must be resolved. Notwithstanding these obstacles, artificial intelligence's prospects for crop breeding appear very bright. Growing progress in AI algorithms, robotics, genomics, and data analytics will allow breeders to create crop varieties that are more nutrient-rich, high-yielding, and climate-resilient. Additionally, breeders and farmers will be able to leverage collective knowledge and expertise through global collaboration and data sharing enabled by AI platforms, which will accelerate the advancement of sustainable agriculture and food security. In conclusion, a major advancement in agricultural innovation has been made with the incorporation of AI technology into plant breeding. With its immense potential to improve environmental adaption and speed genetic selection, artificial intelligence (AI) could completely transform crop breeding. AI algorithms may detect desired features, forecast crop performance, and direct breeding decisions with previously unheard-of precision and efficiency by utilizing enormous volumes of genomic and phenotypic data. In plant breeding, artificial intelligence (AI) technologies are a game-changer. They present hitherto unseen possibilities for tackling urgent agricultural issues and satisfying the needs of an expanding population in a changing climate. Future crop breeding has enormous potential for innovation and worldwide effect as long breeders stick with and improve AI driven technique.

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GENETICALLY MODIFIED ORGANISMS : ADVANCES, APPLICATIONS, AND CONTROVERSIES

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Semester V

ABSTRACT

Genetically Modified Organisms (GMOs) are engineered through advanced genetic techniques to contain specific genes that produce desirable traits, such as increased resistance to pests, enhanced nutritional value, or improved yield. This genetic modification is achieved by isolating the DNA sequences responsible for the desired trait and inserting them into the genome of the target organism. The resulting organisms, commonly plants, animals, or microorganisms, exhibit properties that are difficult to attain through traditional breeding. For instance, Bt corn, a widely used GMO, incorporates genes from *Bacillus thuringiensis* to produce proteins toxic to certain insect pests. This engineered trait reduces the need for chemical insecticides, offering a more environmentally sustainable approach to crop protection.

One of the primary applications of GMOs is in agriculture, where they have been developed to withstand environmental stressors such as drought, extreme temperatures, and herbicides. This resilience allows farmers to cultivate crops under challenging conditions, increasing global food production. Biofortified crops like Golden Rice have also been engineered to provide essential nutrients, addressing vitamin and mineral deficiencies in regions where such deficiencies are prevalent. Golden Rice, for example, has been modified to produce beta-carotene, a precursor to vitamin A, which is crucial in combating vitamin A deficiency – a leading cause of preventable blindness in children in many developing countries.

Introduction

Genetically Modified Organisms (GMOs) have marked a transformative milestone in biotechnology. Defined as organisms whose genetic material has been modified using genetic engineering techniques, GMOs range from crop plants modified for agricultural efficiency to microbes engineered for pharmaceutical production. This journal explores the mechanisms and applications of GMOs, the benefits they confer, and the potential ecological and ethical issues that accompany their use.

Methods of Genetic Modification

Genetic modification involves several methodologies aimed at the insertion or alteration of genetic material within an organism. The most common method is recombinant DNA technology, wherein scientists isolate specific genes with desirable traits and insert them into the genome of a target organism. Techniques such as *Agrobacterium*-mediated transformation, electroporation, and CRISPR-Cas9 gene editing have enabled precise modifications. These modifications can result in phenotypic changes, such as pest resistance, disease resistance, and herbicide tolerance.

Applications in Agriculture

1. Increased Crop Yields and Resistance: Agricultural GMOs have been predominantly developed to improve crop resilience and reduce losses from pests and diseases. Crops such as Bt corn and Bt cotton contain genes from the bacterium *Bacillus thuringiensis*, allowing them to produce proteins toxic to specific insects, thereby decreasing the need for chemical insecticides. Herbicide-resistant crops, such as glyphosate-resistant soybeans, facilitate weed control while minimizing crop damage.
2. Nutritional Enhancement: Biofortified crops aim to address nutrient deficiencies in populations that rely heavily on staple crops. For example, "Golden Rice" was engineered to produce beta-carotene, a precursor of vitamin A, to combat vitamin A deficiencies in parts of Asia and Africa. Similarly, iron-fortified crops are under development to alleviate iron deficiency anemia, a significant health concern globally.
3. Environmental Benefits: The reduced use of pesticides and herbicides in GMO crop cultivation is expected to lessen the environmental footprint of agriculture. By decreasing the frequency of chemical applications,

Medical Applications of GMOs

GMOs have transformed medicine, primarily through the production of pharmaceutical compounds. Engineered microorganisms such as bacteria and yeast have been used to produce human proteins, antibodies, and hormones. Key examples include:

1. **Insulin Production :** Genetically modified Escherichia coli was among the first GMOs developed to produce insulin, revolutionizing diabetes treatment and reducing dependency on animal-sourced insulin.
2. **Vaccine Development :** GMOs are pivotal in producing antigens for vaccines. For instance, genetically engineered yeast produces proteins used in the hepatitis B vaccine, reducing the risk of exposure to live viruses during manufacturing.
3. **Pharming :** In this approach, transgenic animals and plants are developed to produce therapeutic proteins. Goats engineered to express human antithrombin in their milk provide a sustainable source of this protein, crucial for preventing blood clots in certain patients.

Environmental and Health Concerns

While GMOs have undeniable benefits, there are several areas of concern regarding their environmental and health impacts.

1. **Environmental Risks :** The cultivation of GMO crops may affect biodiversity, potentially leading to the development of "superweeds" through gene flow between GMOs and wild relatives. Additionally, monoculture farming practices associated with GMOs can reduce genetic diversity, making crops more vulnerable to disease outbreaks and environmental stresses.
2. **Health Risks :** Although numerous studies have found no significant health risks associated with consuming GMO foods, long-term effects remain a focus of ongoing research. Regulatory

agencies, including the FDA and WHO, continue to monitor GMO safety, with existing GMOs generally deemed safe for consumption. However, allergenicity and antibiotic resistance gene transfer are areas requiring continuous vigilance.

Ethical Implications of Genetically Modified Organisms (GMOs)

The ethical considerations surrounding GMOs are multifaceted and involve questions about environmental stewardship, social justice, food sovereignty, and human rights. These ethical debates often center on humanity's role in altering the genetic makeup of living organisms for human purposes, particularly in agriculture and medicine. The key ethical implications of GMOs include concerns about biodiversity and ecological impact, the rights of farmers and food sovereignty, issues of intellectual property and corporate power, and consumer rights and transparency. Examining these aspects provides a holistic view of the ethical landscape in which GMO technologies operate.

1. Environmental Responsibility and Biodiversity

One of the most pressing ethical concerns related to GMOs is their potential impact on biodiversity and ecosystems. GMOs, especially genetically modified (GM) crops, are often grown in monocultures, where a single crop variety is cultivated extensively. This practice can reduce genetic diversity within crop species, making them more vulnerable to diseases, pests, and environmental changes. Additionally, there is concern about "gene flow," where genes from GMOs can transfer to wild relatives, potentially creating invasive species or "superweeds" that could disrupt natural ecosystems. Ethical debates arise regarding the responsibility humans have to preserve biodiversity and minimize irreversible ecological impacts. Critics argue that introducing GMOs into ecosystems might lead to unforeseen consequences, compromising future generations' ability to benefit from diverse plant and animal species.

2. Food Sovereignty and Farmers' Rights

The control and distribution of GMOs have significant implications for food sovereignty—the right of people to define and control their own food systems. Many GMOs are patented by large biotechnology corporations, which restrict farmers from saving and replanting seeds. Instead, farmers are often required to repurchase seeds each season, creating a dependency on commercial seed suppliers and reducing their autonomy. This issue disproportionately affects small-scale farmers in developing regions, where seed saving is a traditional practice that supports food security and local economies. The question arises whether it is ethically justifiable for corporations to control the resources necessary for food production, especially given the essential role of seeds in sustaining life. Advocates of food sovereignty argue that local communities should have the right to access and control seeds without corporate interference, promoting agricultural practices that are more sustainable and culturally appropriate.

3. Intellectual Property and Corporate Power

Intellectual property rights (IPR) over GMOs present ethical challenges, particularly regarding corporate monopolies on genetically modified seeds. Biotechnology firms invest substantial resources into GMO research and development, leading to patents that legally protect their innovations. However, these patents can concentrate power in the hands of a few corporations, giving them control over a large portion of the global seed market. Ethical concerns arise around the concept of "patenting life," where genetic material—traditionally viewed as a natural resource—is treated as proprietary intellectual property. Critics argue that this practice commodifies life forms and challenges the view of seeds as a shared human heritage. The power imbalance between multinational corporations and individual farmers or local communities also raises questions about social justice, as it may limit farmers' ability to compete and sustain livelihoods independently.

4. **Consumer Rights and Transparency**

   Another key ethical consideration is the right of consumers to be informed about what they are eating and to choose whether or not to consume GMOs. Labeling of GMO products remains a contentious issue, with many consumers advocating for transparent labeling to make informed choices based on health, environmental, or ethical concerns. In the United States, for example, GMO labeling is mandated but often only in specific contexts or via QR codes, which may not be accessible to everyone. In contrast, the European Union has stringent labeling requirements that ensure consumers are fully aware of GM ingredients in their food. The ethical question here is whether consumers have an inherent right to know the origins of their food and whether governments should enforce labeling laws that prioritize consumer transparency. Proponents argue that full disclosure respects consumer autonomy, while opponents suggest that mandatory labeling could unfairly stigmatize GMO products.

5. **Human Intervention in Nature**

   The act of genetically modifying organisms raises broader philosophical questions about the extent to which humans should alter nature for their benefit. Critics argue that genetic modification represents an overreach of human influence, often referred to as "playing God," by artificially altering the genetic code of living organisms in ways that would not occur naturally. This ethical stance suggests that GMOs may undermine the intrinsic value of nature by treating organisms as mere resources to be manipulated. Some ethicists advocate for a precautionary approach, arguing that humans should exercise restraint in altering the fundamental characteristics of other species. Supporters of GMOs, however, counter that genetic engineering is simply an extension of humanity's long history of selective breeding and innovation to improve crop resilience, nutrition, and medicine.

6. **Global Inequality and Access to Technology**

   The benefits of GMOs are not evenly distributed worldwide, and access to GMO technology is often limited to wealthier countries or regions with established biotechnology industries. This disparity raises ethical concerns about equity and justice, as poorer regions may miss out on potential benefits, such as increased food security or access to biofortified crops. Furthermore, because GMO technology often requires significant financial resources and technical expertise, countries or communities without these resources may become dependent on imported GM seeds, exacerbating existing inequalities. Ethical discussions in this area consider whether there is a moral imperative for wealthier nations or corporations to make GMO technology more accessible to developing countries or to support local agricultural development in these regions.

Conclusion

The ethical implications of GMOs are diverse and deeply embedded in the broader context of human rights, environmental stewardship, corporate responsibility, and social justice. Balancing these ethical concerns with the potential benefits of GMOs requires a nuanced approach, recognizing both the technological advances GMOs offer and the potential risks they pose. Moving forward, open public discourse, transparent regulatory frameworks, and responsible corporate practices will be essential in addressing these ethical challenges. Ensuring that GMO technology is used ethically and equitably will involve both respecting the rights and values of diverse communities and carefully weighing the potential impacts on ecological and human health.

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