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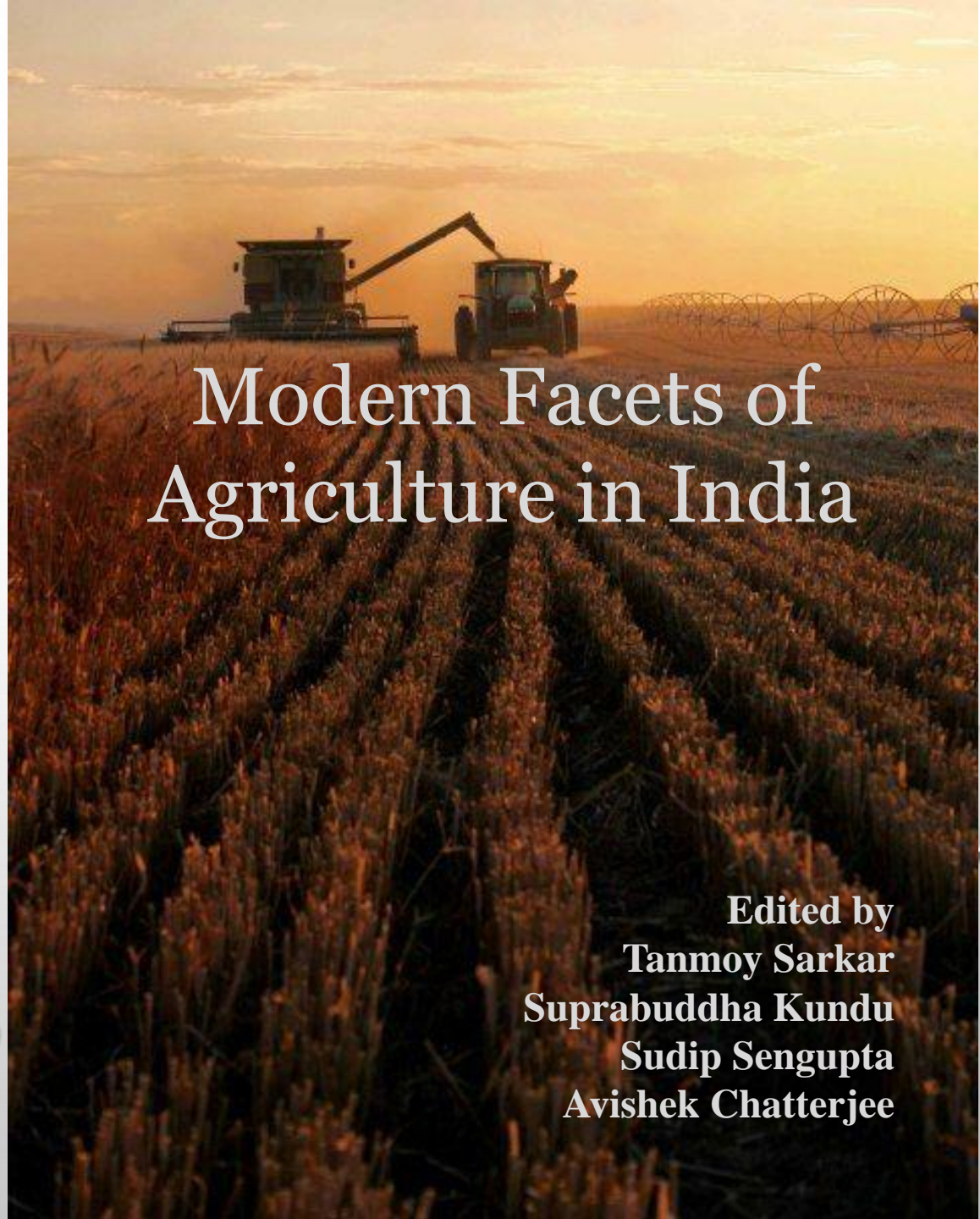


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Modern Facets of Agriculture in India

Sarkar • Kundu
Sengupta • Chatterjee



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Edited by
Tanmoy Sarkar
Suprabuddha Kundu
Sudip Sengupta
Avishek Chatterjee

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Swami Vivekananda University

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PREFACE

In the vast expanse of India's agricultural panorama, the book "Modern Facets of Agriculture in India" serves as a comprehensive guide, delving into an array of contemporary topics that underscore the dynamic nature of farming in the 21st century. The chapters within this volume converge on a spectrum of issues ranging from cutting-edge technologies to sustainable practices, offering an in-depth exploration into the intricate web of challenges and innovations shaping the agricultural landscape of India.

Artificial Intelligence (AI) has emerged as a transformative force in various sectors, and agriculture is no exception. This book opens the door to the realm of AI applications in farming, unraveling the potential it holds in optimizing resource utilization, predicting crop yields, and enhancing decision-making processes for farmers. From smart farming techniques to precision agriculture, the integration of AI is a beacon illuminating the path towards a more efficient and productive agricultural future. Micro-organisms, particularly Plant Growth-Promoting Rhizobacteria (PGPR), take center stage as contributors to soil health and crop productivity. The book provides a nuanced exploration of how harnessing the potential of these micro-organisms can revolutionize agricultural practices, reducing the dependence on chemical inputs while enhancing soil fertility. The sustainability of the rice-wheat cropping system is a pivotal concern, given its prevalence in the Indian agricultural mosaic. This volume scrutinizes the challenges and opportunities inherent in sustaining this crucial cropping system, offering insights into practices that balance productivity with environmental conservation. Diversity, a hallmark of nature, extends to the intricate world of honey bees. The book navigates through the varied species of honey bees in India, emphasizing their role in pollination and the delicate balance required for sustaining biodiversity. Soil water conservation and fertility management emerge as critical components of sustainable agriculture. This volume probes into innovative techniques and time-tested practices that safeguard water resources, enhance soil health, and fortify the foundations of a resilient agricultural ecosystem. As agriculture grapples with the specter of heavy metal contamination in soil, the book investigates the sources, impacts, and remediation strategies. It underscores the urgency of addressing this issue to ensure not only food security but also the long-term health of the environment. Genetic transformation in medicinal plants represents a groundbreaking frontier in agricultural research. As the demand for herbal remedies rises, understanding the genetic makeup of medicinal plants becomes crucial. This book scrutinizes

the strides made in genetic modification, exploring how science is unlocking the therapeutic potential of plants and contributing to the pharmaceutical landscape. The concept of reverse genetics unfolds as a key tool in unraveling the intricacies of plant biology. This book sheds light on how this approach is reshaping our understanding of plant genetics, enabling scientists to modify genes for improved crop traits, disease resistance, and environmental adaptability.

"Modern Facets of Agriculture in India" is a compendium that invites readers on a journey through the contemporary nuances of Indian agriculture. It is a testament to the tireless efforts of researchers, scientists, and farmers striving to strike a harmonious balance between technological advancements and sustainable practices. As we traverse the diverse landscapes of AI, genetic transformation, sustainability, and beyond, this book serves as a beacon guiding us toward an agriculturally resilient and environmentally conscious future.

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ABOUT THE BOOK

“Modern Facets of Agriculture in India” stands as a comprehensive exploration, delving into the intricate tapestry of contemporary agricultural practices within one of the world's most agriculturally diverse nations. This book encapsulates a dynamic panorama, navigating through topics that define the cutting edge of agricultural innovation. From the strategic infusion of artificial intelligence into farming practices to the microscopic world of PGPR micro-organisms, the sustainability of crucial cropping systems, the nuanced management of soil fertility, the biodiversity of honey bees, soil water conservation, and the pervasive issue of heavy metal contamination in soil. Additionally, the book unravels the transformative potential of genetic transformation in medicinal plants and concludes with the revolutionary approach of reverse genetics in crop improvement.

At the forefront of this exploration is the incorporation of artificial intelligence (AI) into the fabric of Indian agriculture. AI, with its capacity for data analysis, predictive modeling, and automation, presents a transformative paradigm shift. The book meticulously examines how AI is reshaping traditional farming practices, offering solutions that optimize resource allocation, enhance decision-making processes, and propel the agricultural sector into a more efficient and sustainable future. In a country facing the challenges of feeding a burgeoning population, the infusion of artificial intelligence emerges as a beacon of innovation, promising to revolutionize the landscape of Indian agriculture.

The microscopic realm of Plant Growth-Promoting Rhizobacteria (PGPR) micro-organisms takes center stage as contributors to soil health and crop productivity. This section of the book unravels the often-overlooked symbiotic relationship between these micro-organisms and plants. By promoting plant growth, improving nutrient uptake, and fostering soil health, PGPR micro-organisms become instrumental in sustainable agriculture. The book showcases real-world applications, illustrating how harnessing the potential of these micro-organisms can reduce reliance on chemical inputs, providing an environmentally friendly alternative for farmers.

The sustainability of cropping systems, particularly the rice-wheat system, is critically examined within the pages of this volume. This integral component of Indian agriculture faces challenges from changing climatic conditions, soil health concerns, and environmental impacts. The book delves into innovative practices that seek to sustain agricultural productivity while minimizing ecological footprints. From agro ecological approaches to crop diversification, the exploration extends to strategies that balance the imperative for food

security with the preservation of the ecosystems supporting these staple crops.

Soil fertility management becomes a linchpin in the pursuit of sustainable agriculture, and the book navigates through the myriad strategies employed across India. The chapters explore organic farming practices, precision fertilizer applications, cover crops, and crop rotation—holistic approaches aimed at maintaining nutrient levels in the soil. This exploration provides a nuanced understanding of the intricate dance between traditional wisdom and modern scientific practices. The symbiosis between age-old agricultural practices and contemporary science emerges as a cornerstone in the book's narrative.

Biodiversity, often underappreciated in the context of agriculture, takes the spotlight with a dedicated exploration of honey bees. The book celebrates the rich diversity of honey bee species in India, emphasizing their indispensable role in pollination. Beyond their role as honey producers, honey bees are recognized as vital contributors to biodiversity and food security. The chapters delve into the challenges faced by honey bee populations, underscoring the need for conservation efforts to ensure the continued interdependence between agriculture and natural ecosystems.

Soil water conservation becomes imperative in the face of increasing water scarcity. The book probes into a spectrum of innovative techniques and time-tested practices aimed at preserving and efficiently using water resources in Indian agriculture. From traditional rainwater harvesting to advanced precision irrigation methods, the exploration encompasses a range of approaches to address the challenges posed by water scarcity. The imperative of optimizing water usage in agriculture resonates as a critical component of sustainable land management.

Addressing the contemporary concern of heavy metal contamination in soil, the book investigates the sources, impacts, and potential remediation strategies. This exploration extends beyond agriculture to touch on environmental and public health considerations. The urgency of addressing heavy metal contamination becomes apparent as the chapters unfold the multifaceted challenges and potential solutions. The interconnectedness of agricultural practices, industrial activities, and environmental health takes center stage in this section.

The exploration culminates in the intricate realms of genetic transformation in medicinal plants and the revolutionary approach of reverse genetics in crop improvement. These sections unravel the potential of manipulating plant genetics to enhance medicinal properties, understand gene functions, and engineer crops for desired traits. The chapters showcase the transformative potential of genetic innovations in reshaping the agricultural landscape. The ethical considerations and societal implications of manipulating genetic

information become integral components of these thought-provoking explorations, emphasizing the need for responsible and sustainable agricultural practices.

In essence, "Modern Facets of Agriculture in India" offers a panoramic view of the dynamic interplay between tradition and innovation. It is not merely a compilation of topics; it is a narrative that unveils the intricate dance of nature, science, and tradition in the fields that sustain a nation. The book stands as a testament to the resilience and adaptability of Indian agriculture, navigating the complexities of modernity while preserving the essence of sustainable and resilient farming practices.

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CHAPTER 1

Artificial Intelligence: an Advance tool for the Agriculture-Food Industries

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Abstract:

Artificial intelligence (AI) is the process of creating computational models and algorithms that let robots collect and analyze vast volumes of data, spot trends and links, and then forecast or decide what to do next. In a variety of sectors and industries, including healthcare, banking, manufacturing, retail, education, and agriculture, artificial intelligence (AI) is becoming more and more prevalent. The influence of AI technology on sectors is predicted to increase in the future as it develops.

Keywords: agriculture, robots, intelligence, productivity

Introduction

The population of the globe is predicted to increase quickly, reaching approximately 9.7 billion people by 2050. The question of how to provide the growing demand for food while maintaining food security and sustainability is therefore becoming more and more pressing. This is why the agri-food business might undergo a revolution and become more sustainable in a number of ways through the use of artificial intelligence (AI) applications. It can enhance food security and sustainability, increase efficiency, decrease waste, and assist farmers, food producers, and distributors make better decisions. In 1965, the economist Herbert Simon, who won the Nobel Prize, predicted that machines will be able to perform every task that a human could. The amazing advancements made possible by AI applications today are a testament to his imaginative viewpoint (Pournader et al., 2010). Artificial Intelligence (AI) is the ability of computers or computer programs to carry out tasks like learning, reasoning, problem-solving, and decision-making that often require human intelligence.

Artificial Intelligence (AI) has many subfields, such as computer vision, robotics, natural language processing, machine learning (ML), deep learning, and cognitive computing. Artificial Neural Networks (ANN), Logic Programming, Swarm Intelligence, Fuzzy Logic (FL), Expert Systems, Reinforcement Learning (Pantazi et al., 2016), and Cognitive Science are a few algorithms that can be employed in AI technology (Kumar, and Thakur, 2012). The

selection of an algorithm is contingent upon the particular task or issue at hand, as each algorithm possesses distinct benefits and drawbacks. AI is being employed in many different fields, including financial forecasts, autonomous vehicles, image and video analysis, speech recognition, and medical diagnostics. AI may be applied in the agri-food sector, just like it can in any other business, to increase productivity (Xiong et al., 2020), create new, more nutrient-dense crops, cut waste, and guarantee safety. AI can be utilized to enhance distribution and logistics, as well as maximize crop yields.

Artificial intelligence (AI) can be used in precision agriculture to evaluate data from sensors, drones, and satellites to maximize agricultural techniques including fertilization, irrigation, and pest control. Higher yields, lower prices, and a smaller environmental effect are possible outcomes of this. AI-powered cameras and sensors for agricultural monitoring are able to track crops in real time and identify pests, illnesses, and nutrient shortages. This enables farmers to avoid crop loss and act swiftly. To forecast agricultural yields and market demand, artificial intelligence (AI) systems can examine historical data, soil conditions, and weather patterns. This can assist farmers in scheduling their planting and harvesting operations and in maximizing their pricing plans (Talaviya et al., 2020). Artificial Intelligence (AI) has the potential to optimize supply chains through waste reduction, demand prediction, and logistical optimization. AI algorithms, for instance, can be used to route trucks, forecast the best time to harvest crops, and manage inventory levels. AI can be applied in the food processing sector to identify flaws or pollutants in food products and to improve food processing tasks like grading and sorting (Kakani et al., 2022).

Fruits and vegetables can also be sorted and identified using AI according to characteristics like size, color, and other characteristics. This can lessen waste while also enhancing the uniformity and quality of food products. By evaluating data from sensors and cameras to find possible pollutants or other risks, artificial intelligence (AI) can be used to monitor food safety. This can enhance public health by preventing foodborne sickness. Furthermore, AI can be utilized to deliver customized dietary recommendations by analyzing customer data such as age, gender, and activity level. This can enhance general health and enable customers to make better-informed dietary decisions. Even if these studies offer some insightful information on AI applications in the agri-food industry, a thorough analysis is still required to comprehend the most recent developments in AI technology in this field. Thus, the goal of this analysis is to draw attention to the latest advancements in the food and agriculture industries as well as the use of AI technology, with particular examples drawn from databases

between 2010 and 2023. The review also provides an overview of the field's constraints, difficulties, and future possibilities.

The Application of AI in Agriculture

Since its inception, the food business has always been reliant on the agricultural sector. The Fast-Moving Consumer Goods (FMCG) businesses, which depend on these raw materials for product processing and manufacture, may have access to a greater supply of raw materials if the farm sector increases food production (Sharma et al., 2020). Numerous lives have been profoundly impacted by the COVID-19 epidemic, and the supply of various sectors has been negatively impacted. Many industries throughout the world were forced to close as a result of the government's decision to proclaim a state of emergency, which affected the whole supply chain from the farmer to the consumer (Sridhar et al., 2023). The COVID-19 pandemic is linked to a number of factors, including the unanticipated loss in output and revenue, the decline in oil prices, the decline in tourism receipts, the problems with climate change, and other factors. The FAO reports that there has been an increase in the number of persons experiencing hunger and malnutrition in recent years. However, the agriculture sector can address many issues affecting crop productivity and increase the amount and quality of raw materials accessible to the food industry by implementing AI and ML in crop management and employing sophisticated automated systems. This section discusses some of the machine learning (ML) technologies that have been used in the agriculture sector and have helped to improve crop management.

Pest Detection and Weed Management

Effective pest management in agriculture depends on the accurate identification of insect species, size variation, and developmental stage. Farmers can take the necessary action to manage the pest population and avoid harm to their crops by determining the kind and quantity of insects present in a particular area. A number of AI and ML systems are being researched and tested for the purpose of detecting and counting insects. While some of these technologies classify and identify various insect species using computer vision techniques, others employ machine learning algorithms. It's crucial to remember that these technologies are still in the testing phase and haven't been extensively incorporated into the agriculture sector yet. In a similar vein, farmers have long employed herbicides to reduce weeds and increase crop yields. Nevertheless, using herbicides excessively or incorrectly can have detrimental effects on the environment and human health. More exact and accurate spraying techniques are becoming more and more necessary to reduce the harmful effects of

herbicides. Herbicides can be applied only where necessary by farmers using precision agricultural techniques like site-specific application, which lowers the amount of chemicals required and lowers the chance of contamination. The creation of AI-based tools that identify and categorize various weed species in agricultural fields using machine learning and computer vision techniques has the potential to increase agricultural productivity and sustainability while lowering the need for herbicides and raising crop yields.

Another cutting-edge technology that has enormous potential for the future of agriculture is robotic weed control. Robotic weed control systems generally employ robotic arms or other mechanical equipment to remove or eliminate weeds in crop fields after detecting and identifying the weeds using computer vision and machine learning techniques. These systems can be used in a variety of agricultural settings, such as greenhouses, where conventional weed control techniques like pesticides would not be suitable or successful. For both intrarow and interrow weed control, cultivars may be fitted with elastic tines or finger weeders (Christensen et al., 2009). Precision weed management, a component of precision farming, is based on the use of information technology to analyze site-specific weed control (Wu et al., 2020). In contrast to time-based weed removal, intelligent mechanical weed control would be more beneficial than weeding devices with cutting action (Rasmussen 2012). However, the prototype spring-tine harrow systems' tine tendency can be remotely adjusted based on crop productivity, soil conditions, and weed density.

Selection of Crop and improvement of yield

Global food security is significantly influenced by agricultural planning, particularly in nations where the economy is agro-based. Choosing appropriate crops with higher yields is a difficult task since many factors can affect them, including the climate, the quality of the soil, the availability of water, pests, and illnesses (Dhanaraju et al., 2022). In agriculture, the application of AI and ML technologies to crop selection and production enhancement is growing. Genetic improvement and crop breeding benefit greatly from these methods. Plant breeders can create new crop varieties that are more suited to a given environment and yield more by utilizing machine learning (ML) algorithms to analyze genetic data from various crop types and uncover important factors linked to yield and other desirable attributes. An increasing number of agricultural operations, such as weed removal, pesticide spraying, and fruit and vegetable harvesting, are being done more efficiently and profitably with the use of automation technologies, such as robots. Harvesting strawberries is a labor-intensive and time-consuming task that can be automated with the use of robots as Harvest Croo Robotics'

Berry 5 Robot (Tampa, FL, USA) (Xiong et al., 2020). The robot can identify and select ripe strawberries more quickly than people by using computer vision and machine learning algorithms. This ensures that more strawberries are collected at the best moment, which can help growers increase yields and save labor expenses.

Application of Artificial Intelligence in Food Industry

The food processing business is progressively embracing AI and ML technology. These technological advancements are contributing to the improvement of overall efficiency, quality control, and process optimization. Along with the products and equipment created by various AI technologies, the abilities of intelligent systems in a variety of tasks including intelligent food packaging, product sorting, foreign object detection, the development of new food products, equipment cleaning, and supply chain management are explained.

Sorting of Products

In order to assess product parameters at the input level, AI-based systems can integrate a range of technologies, including laser technology, X-ray systems, high-resolution cameras, and infrared (IR) spectroscopy (Cai et al., 2020). By assisting in the identification of flaws, pollutants, and irregularities in the items, these technologies can help make intelligent decisions and raise the general caliber of the output. However, sorting techniques that depend on input-level evaluation may suffer greatly from the uneven product homogeneity. Products that are not uniform may lead to poor sorting choices, which could increase waste or degrade the quality of the final product. Some sorting systems address this issue by utilizing a variety of sensors or technologies to evaluate the quality of the product and spot flaws from various angles. Furthermore, by training ML algorithms to identify trends and variances in product attributes, sorting decisions can become more accurate and consistent. The world leader in sophisticated gathering and sorting systems, TOMRA (Asker Municipality, Norway), has created an AI system that can sort data 90% of the time. Industries have benefited from using such systems in a number of ways, including higher yields, lower labor costs, and expanded production. According to reports, the problems with potato segregation and layout can worsen by 5% to 10%.

Recognizing Foreign Objects

Foreign object contamination results in serious problems, such as food recalls, consumer rejection, customer injury, and a decline in the trustworthiness of the brand. Insects, glass, metal, or rubber are examples of foreign objects that could inadvertently find their way into food during processing, handling, or preparation. Eating food contaminated with foreign

objects can cause choking or other issues, even though the dangers connected with them vary depending on their size, kind, clarity, and hardness.

Since it can be difficult to identify these contaminants with the untrained eye, artificial intelligence (AI) and machine learning (ML) technologies can play a significant role in their detection by evaluating photographs of the food products to identify any potential foreign objects (Sabanci et al., 2016). Using image recognition algorithms that have been trained on sizable datasets of photos of tainted and uncontaminated food products is one method for detecting foreign objects using AI and ML. Then, using real-time picture analysis of the food goods, the system can detect any potential foreign objects.

Food Product Development

Since the food business entirely depends on customer viewpoints for new product creation, the information gathered by the various decision-making systems is helpful in the introduction of novel goods. After examining the information that the system collected, the ML-based module might provide a response to the query "what exactly are the consumers looking for" and make appropriate choices. A global corporation has implemented automated vending machines. There are thousands of soft drink delivery machines in the USA for customers to choose from. many choices to choose their preferred flavors

Cherry Sprite, a new product the company launched, is one example of how the data recorded by the machine could be examined by the ML module and deep learning algorithms. Additionally, it has been suggested that many of the food businesses would profit from the ML-based decision-making system in the next decades when it comes to the introduction of new food products. Using AI technology, a biotechnology company has introduced the first bioactive peptide in history. A special peptide network made from rice protein is used as an element in sports nutrition to improve immune function and reduce inflammation by modifying cytokine responses. The business is now the first in the world to show how artificial intelligence (AI) may be used to improve human health. An additional US-based IoT technology company has released AI-powered "home cooking sidekick," a mobile and web application that pairs with Hello Egg, a smart kitchen helper, to completely automate household chores. The home assistant uses voice technology to suggest a diet program depending on each user's preferences. Additionally, it can organize the shopping cart, maintain the pantry, provide recipes in videos, and help with grocery delivery. The purpose of sensory panelists working in the food and beverage industry is to assess new goods using their senses in accordance with consumer preferences for flavor. Regretfully, it is challenging to forecast how the target group will be perceived and what their preferences will be. Due to

this, the industry created a reliable methodology for gauging and forecasting consumer preferences using the AI-based Gastrograph system, which interprets market choice using machine learning and predictive algorithms (Trencher, 2019).

Technology for 3D/4D Food Printing Extrusion

Artificial intelligence (AI) can be used to streamline production, lower mistake rates, and improve printer performance in 3D and 4D printing. The fusion of artificial intelligence (AI) with 3D/4D printing technology has the potential to create new businesses and research initiatives that incorporate AI into 3D/4D printing goods and services. Globally, the food processing industries are currently implementing 3D printing technology to provide more autonomous and methodical processes. By enabling computerized food customisation, lowering food waste, and facilitating on-demand food manufacture, this technology can make active food value chains more profitable and customer-friendly.

The main distinction between a standard 3D printer and a 3D/4D food printer is the type of printing media that is utilized. A 3D/4D food printer employs food materials as the printing medium rather than melted plastic. Customers can use websites or mobile applications to materialize designs from an e-commerce platform, which reduces the cost of warehousing, packaging, and delivery. By perfecting 3D printing methods and tools, food printing becomes more efficient while food processing costs and duration are reduced (Nachal et al., 2019). Additionally, time is saved. Another benefit is that 3D printing technology will enable personalized items to be delivered faster than traditional food processing methods. Food safety will also be improved because fewer synthetic polymer-based packing materials and chemical preservatives may be required as a result of the products being delivered considerably faster. Moreover, food product multiphase processing could be reduced to a single step.

Application in Food quality and food quality safety

AI is a very attractive technology to apply in industries for decision-making, process estimates, cost reduction, high profitability, and overall quality enhancement because to its fascinating capabilities. AI and data science together have the power to raise customer satisfaction, revenue, and profitability by enhancing the food and service provided by food stores, restaurants, and online meal delivery services. Artificial intelligence (AI) may enhance supply chain efficiency, menu optimization, sales forecast, and tailored suggestions by evaluating data and using algorithms. AI has significantly improved the food sector in a

number of areas, and these improvements fall into three primary categories: food waste control, food security management, and food quality management.

Artificial Intelligence (AI) has the potential to optimize production and enhance product quality by analyzing vast volumes of data from dairy production operations and identifying trends and forecasts. Dairy farmers can achieve increased efficiency and superior quality of production by employing artificial intelligence (AI) techniques like fuzzy logic and neural networks (ANN) to generate more precise predictions and modify their procedures accordingly. In the beverage sector, artificial intelligence (AI) solutions like e-nose, e-tongue, CVS, and picture analysis can assist producers in streamlining their operations and comprehending the preferences of their customers. Additionally, AI may aid the bakery sector by boosting efficiency and improving product quality. Bakeries can also expand their creative output and streamline their manufacturing processes by utilizing AI-powered tools like robots and visualization.

Management of Food Quality

Fresh produce is extremely perishable and can go bad fast if improperly stored and handled. A substantial amount of food was wasted in the past because many merchants lacked the equipment needed to keep an eye on the condition of fruits that were being stored in real time. That being said, a number of technologically advanced technologies are now accessible to assist vendors in tracking the state of fresh produce in real time. A promising technique for keeping an eye on the quality and state of fresh produce along the cold chain is digital twin (DT) technology. Fruits' tissue damage is influenced by a number of variables, including their physical and chemical characteristics as well as environmental elements including humidity, temperature, and postharvest handling. By keeping an eye on these variables with DT technology, possible problems can be seen early and remedial action can be taken to stop more harm from occurring.

DT technology can be utilized to enhance fresh fruit and vegetable storage and transportation, in addition to monitoring the cold chain (Gowen et al., 2010). In the fruit and food sector, thermal imaging is a non-contact, developing technology that is gaining popularity for evaluating fruit quality. It provides a non-destructive method of product examination instead of extraction, which can result in irreversible harm. By detecting the temperature differential between undamaged and damaged tissues—a result of variations in thermal diffusion coefficients—infrared thermal imaging can identify the existence of damage or flaws in fresh fruits and vegetables (Gowen et al., 2010). Similarly, the food business is using CVS technology more and more to assess the quality of various food items. It is feasible to spot

probable problems early on and take corrective action to stop further degradation or contamination by employing several forms of CVS, such as classic CVS, hyperspectral CVS, and multispectral CVS, to assess the external quality of food goods.

In the food market, ethernet technology (ETS) holds great potential for evaluating the characteristics of many beverage kinds, such as dairy and alcoholic ones. Ets are capable of identifying a variety of flavor attributes, including sweetness, saltiness, sourness, and bitterness, all of which can play a significant role in assessing a product's overall quality. For instance, different taste components, such theaflavin (TF) and thearubigin (TR), have different concentrations depending on how old the tea. These chemicals may be detected and used to determine the type of tea being examined by combining a UV-VIS spectrophotometer-based analysis with a pulse voltametric ET.

Management of Food Safety

Food safety guarantees that it satisfies the mandatory nutritional criteria and is free of any hazardous or poisonous ingredients. To guarantee food safety and proper hygiene throughout food processing, storage, and sale, as well as to reduce the possibility of biotic and abiotic pollutants that might result in food poisoning, a multidisciplinary approach is required.

Food product attributes such as size, shape, color, and texture may all be analyzed using image processing methods. One may quantify the size and form of food products by calculating their predicted area and perimeter, which can be helpful for quality control purposes. It is important to remember, nevertheless, that image processing methods cannot identify dangerous microbes or other possible threats to food safety on their own. This is the application of next-generation sequencing (NGS), also known as "whole genome sequencing" (WGS) and "metagenomics," which uses NGS to produce sequences of multiple microorganisms in a biological sample. NGS is utilized to determine the whole genome sequence of a single cultured isolate (such as a bacterial colony, a virus, or any other organism). Additionally, the analysis of NGS data may be completed much more quickly with the assistance of AI and automation, which makes it possible to identify any food safety problems earlier. This makes it possible to act quickly to stop the spread of sickness before tainted items are consumed by customers.

Management of Food Waste

Food waste is a serious problem that has an impact on the environment, food security, and the viability of the economy. An estimated 1.6 billion tons of food are wasted yearly, with inedible byproducts of food production processes accounting for the majority of this loss (81%) of food. Within the food sector, there is a growing realization that food waste is not

only an inevitable expense of doing business but also a major sustainability concern and a resource that is underused (Tavill, 2020). By lowering food waste by 2030, artificial intelligence (AI) might present a \$127 billion opportunity, according to McKinsey & Company (New York, NY, USA), a consulting firm that has been at the forefront of investigating and deploying AI in numerous sectors. omics is one of the modern methods that may be used to tackle difficulties related to food waste management and reduction. To discover possible pollutants and risks in food waste, for instance, metagenomics, proteomics, transcriptomics, waste omics, and disease omics may be utilized to comprehend the biochemical processes that occur during food waste breakdown.

To lessen and control the problems associated with food waste, experts and government agencies have created and tried a number of various concepts and solutions. For example, Black Soldier Fly (BSF) farming is a promising technique because of its adaptability and several uses, including managing food waste and producing sustainable food. In terms of effective production and waste management, BSF farming may greatly benefit from the use of Internet of Things (IoT) technologies. Farmers may monitor and manage several aspects of BSF farming remotely by combining software, smartphone applications, and sensors and equipment. This enables more accurate and tailored control over the BSF larvae's growth environment, resulting in increased biomass yields and higher-quality biomass.

Shelf Life Predict

Food shelf life has been frequently predicted using AI and ML approaches, such as ANNs. These methods create prediction models by utilizing data from several sensory and physicochemical aspects of the food product along with mathematical models. That is capable of estimating anticipated shelf life (Ferreira and Goncalves, 2022). In a research study, Goyal and Goyal, (2012) suggested using time-delayed neural network (TDNN) models to forecast processed cheese's shelf life. The goal of the project was to create a model that would be able to forecast processed cheese's shelf life with accuracy and eliminate the necessity for labor-intensive physical testing. The study's findings demonstrated that the TDNN model could correctly forecast processed cheese's shelf life. AI models based on respiration rate and ripeness levels under various supply chain scenarios were created to forecast the shelf life of mangoes stored under various settings. Using 1524 picture data of mangoes, a deep neural network was optimized to classify the various ripeness degrees of mangoes.

Conclusions and Future Perspectives

The creation and application of AI technologies, the laws and rules governing their application, and how society responds to the changes AI brings about will all have an impact on how sustainable AI becomes. AI sustainability takes into account a number of social, cultural, and environmental aspects. When it comes to the sustainability and future of AI, there are a number of important factors that must be taken into account. In order to develop and implement AI systems in a sustainable and ethical way, it is imperative to address the skills gap and guarantee that there is a sufficient pool of expertise. This necessitates funding educational and training initiatives that can provide people with the abilities and information need to work.

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CHAPTER 2

Perspectives and challenges of plant growth promoting microbes as biocontrol agents

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Abstract

Pests and plant diseases are risk factors that jeopardise the security of food worldwide. Overuse of chemical pesticides is a typical strategy to mitigate the impacts of bacterial and fungal pathogen-caused plant diseases. A key priority in our pursuit of a more sustainable agriculture is raising agricultural yields to feed the growing population. The efficiency of microbial biological control agents (MBCAs) as an environmentally friendly method of managing plant diseases, promoting growth of plants, and boosting output has been demonstrated. Plant growth-promoting rhizobacteria/fungi (PGPR/PGPF) have the potential to lower down plant diseases in addition to enhancing growth. They do this by generating compounds that limit plant development and stimulating plant defences against phytopathogens. PGPR and PGPF, as biofertilizers and biopesticides, are seen as a viable, economic strategy for sustainable agriculture. A number of strains of PGPR and PGPF are found to function as efficient BCAs in controlled environments.

Keywords: Biocontrol agents, Plant growth promoting rhizobacteria, fungi

Introduction

Pests and plant diseases may significantly affect how productive agriculture is. Plant infections cause output reductions of 21-30% in a number of globally significant crops. In the meanwhile, several plant infections have become resistant to chemical control over time. Certain plant diseases that are significant commercially have increased in frequency. Using chemical pesticides is one of the biggest threats to human health and environment worldwide. Hazardous residues stay in the soil, raising questions about health, because many pesticides are difficult to decompose into less hazardous, simpler components. Sustainable crop management and decreased usage of chemicals need an understanding of the threats to the environment and human health posed by synthetic chemical pesticides.

Since synthetic agrochemicals are seen to be unsustainable, there is a search for more ecologically benign substitutes. Farm practises are now the main focus of study in modern

agriculture. Natural, non-toxic, and ecologically friendly microorganisms called plant growth-promoting rhizobacteria (PGPR) is capable to be a viable substitute for conventional pesticides. Furthermore, agricultural output can be impacted by environmental conditions, which might exacerbate the situation in a number of ways. To enhance our health and lessen the consequences of environmental stressors, there are several reasons why we should take proactive measures to minimise plant diseases (Chaloner et al. 2021).

A thorough scientific analysis of the link between the characteristics of plant growth-promoting microorganisms (PGPMs) along with their effects on crop yield, growth and resistance to biotic and abiotic challenges is necessary in light of recent developments in our understanding of PGPMs. Furthermore, this review study adds to the expanding body of research on certain possible applications of PGPMs in sustainable agriculture.

Role of PGPR as a biocontrol agent (BCA)

Bacteria, fungi, protists, and mammals are just a few of the diverse kinds of microorganisms found in the complex ecosystem that is soil. These microorganisms are necessary for plant development, nutrition management, and biocontrol mechanisms. They get established in the plant's the root system, where they use a variety of techniques to advance plant growth. According to Lyu et al. (2020), plant-associated microorganisms, or phytomicrobiome, can form cooperative, exploitative, or competitive relationships with plants, which can have an impact on crop productivity. In order to suppress phytopathogens and encourage plant development, scientists have recently focused extensively on the use of beneficial PGPR. Numerous bacteria found in the rhizosphere supply consistent PGPR. Bacteria that lives in the root system, on the surface of the roots, and in the spaces between the root cortex cells is referred to as the phytomicrobiome. Plants have evolved simultaneously with related plants since they can initially inhabit terrestrial habitats, leading to connections between PGPR and their host plants that are synergistic (El-Sadoony et al. 2002).

Vegetables are used mostly unprocessed or less processed and have an influence on human health, and quality control are important in farming of vegetables. A greenhouse environment makes PGPR more attainable. Their success in greenhouse investigations has been validated, as a large number of potential BCA have been found and may be ready for implantation because to the controlled environment. Three bacteria have been found to be efficient- in both root colonisation and preventing the disease *Phytophthora capsici* in cucumbers: *Bacillus subtilis*, *Bacillus amyloliquefaciens*, and *Pseudomonas stutzeri* (Islam et al., 2016). Tomato fruits can be shielded from infection by *Penicillium* sp. and *Rhizopus stolonifer* during the

post-harvest stage by *B. subtilis*. *B. amyloliquefaciens* isolates reduce *Fusarium oxysporum*, which causes *Fusarium* wilt disease, in greenhouse environments.

The synthesis of plant hormones, phosphate solubilization, and enhanced iron absorption are examples of direct methods. The generation of antibiotics, nutritional rivalry, parasitism, pathogen toxin inhibition, and induced resistance are examples of indirect effects. Additionally, PGPR may change the root's morphology, increasing its surface area and enhancing its functionality. Furthermore, by colonising quickly and accumulating a larger source of nutrients than other bacteria, PGPR can compete with them to stop other species from flourishing.

Challenges of PGPR as biocontrol agents

New biocontrol agents have been developed from researches conducted in the United States and Europe. PGPR was studied in lab and also commercially confirmed as BCAs (Rosier et al., 2018). Although the market for BCAs and their items are expanding overall, chemical pesticides are still the most often used crop management technique, and BCA adoption is lower.

There are prerequisites and demands that must be met before a commercial BCA is officially recognised or registered. Therefore, studies should be conducted to increase BCAs' effectiveness in treating specific diseases. This may be accomplished by designing a BCA with many advantageous traits and modes of operation as feasible. These traits could include, but are not restricted to, the BCA's capacity for rapid in vitro growth, production of a broad variety of bioactive molecules, high competition in rhizosphere, improvement of plant growth, safety for the environment, compatibility with other microbes, and resistance to abiotic stresses (Lyu et al., 2020). A necessary condition for a PGPR strain to be a potent BCA and effectively combat plant diseases is the colonisation of root tissues. However, the inoculated PGPR's performance might differ based on the soil survival rate, crop suitability, interactions with other nearby microbial species, and climatic conditions. For any PGPR strain to be a potent BCA and effectively combat plant diseases, it must successfully colonise root tissues and/or the rhizosphere. Conversely, the efficacy of the injected PGPR may differ based on the rate of survival in soil, crop suitability, interactions with other nearby microbes, and environment.

The procedure, formulations, shipping, and storage circumstances all have an impact on PGPR stability. In order to attain elevated BCA survival levels, it is recommended to enhance formulation technology, prolong the BCA product's shelf life, maximise the production of

specific microbial types, and attain cost-effective production in a large scale (El-Sadoony et al. 2022). Begum et al. (2017) have cited high development costs for new commercial BCAs as one obstacle to the BCA industry's growth in Australia. One of the biggest obstacles is the significant regulatory costs associated with bringing new BCAs into Australian markets. To support the evaluation and marketing of new BCAs and their products, BCA registration necessitates close collaboration between governmental agencies, academic institutions, and business sectors. The lack of initiatives with both financial and environmental advantages might be mentioned as another difficult issue (Heimpel et al. 2013).

Role of Rhizobacteria as biocontrol agent

Rhizobacteria have gained interest in recent decades when they are added to seeds, grains, roots, and/or soils to aid in plant growth and development. Rhizobacteria have a major role in N₂ fixation, plant growth promotion, and the control of pathogenic microbes biologically that affect plants. *Acinetobacter*, *Agrobacterium*, *Alcaligenes*, *Arthrobacter*, *Bradyrhizobium*, *Frankia*, *Pantoea*, *Pseudomonas*, and *Thiobacillus* are among the microbial species that are currently employed in bacterization (Whipps, 2001). Other species include *Azospirillum*, *Azotobacter*, *Bacillus*, *Rhizobium*, *Serratia*, *Stenotrophomonas*, and *Streptomyces*. Numerous plant diseases linked to bacterial, fungal, and nematodial infections have been documented as being treated by PGPR.

A strain of *Pseudomonas* sp. strain grown in vitro culture filtrates has the ability to inhibit *Meloidogyne javanica* juvenile mortality, significantly reducing the number of nematodes and root galls while also improving plant growth and yield. Moreover, nematode behaviour and feeding are impacted by *Bacillus* species inoculations. The nematode population is also disrupted by isolates of *B. subtilis*, *Pseudomonas fluorescens*, and *Pseudomonas striata*. Biological treatment with *Bacillus* isolates has shown to be successful in controlling infestations of root-knot nematodes (El-Sadoony et al. 2022). Various compounds produced by *Bacillus* spp. aid in the control of phytopathogens biologically on a range of plants, such as cucumber, rice, tomato, groundnut, brinjal, and wheat (Peng et al., 2014). Grey mould and powdery mildew diseases in strawberries and cucumbers can be less common when *Bacillus* sp. BS061 isolate is used to counteract the effects of *Botrytis cinerea*.

Fungi as agents of biocontrol

Plant diseases can be repelled by fungus-based BCAs, which also shield the host plants. To combat the fungal infections *Penicillium*, *Fusarium*, *Aspergillus*, *Alternaria*, *Pythium*,

Rhizoctonia, *Phytophthora*, *Pyricularia*, and *Botrytis*, for instance, a number of strains of *Trichoderma* have been released as BCAs. *Trichoderma*, being a BCA, has the ability to inhibit a variety of soil- and air-borne plant diseases; as such, it might be employed as a biopesticide in field or greenhouse experiments. It was discovered that in soils grown with tomato plants, the talc-based formulation of the fungus BCA, *Paecilomyces lilacinus*, was more effective in lowering the numbers of *Meloidogyne incognita*. It has been noted that *P. lilacinus* effectively suppresses nematodes in a number of horticultural crops, such as capsicum, okra, and tomatoes. *P. lilacinus* lowers the population of *M. incognita* on the roots and in the soil, it can increase tomato production (Kalele et al. 2010). The nematode density in the soil was significantly decreased by seed coating with *P. lilacinus* and *Trichoderma viride*. When compared to the single bio-agent treatment, it was shown that *Aspergillus* and *Paecilomyces* species were hostile to *M. incognita*, which led to increased plant growth. Okra seeds treated with *Trichoderma harzianum*, *T. viride*, *P. lilacinus*, *P. chlamydosporia*, and *P. fluorescens* at 20 g kg⁻¹ seed significantly reduced the nematode population in the soil and promoted plant growth development.

Competition of MBCAs within the rhizosphere

When a colony of a certain microbe strives for something bigger, like space or food supply, coexistence of two live microorganisms takes place. In the root system, pathogenic and non-pathogenic microbes fight it out due to resources and nourishment. Long recognised is the fact that non-pathogenic bacteria associated with plant are often shielded by the plants they colonise, which weakens the few substrates that are accessible and stops the pathogens from spreading. Rhizosphere competence is the capacity of any bacterium to invade the roots of host plants and compete with other microbes for nutrition and exudates released by the roots of plants. Microbial communities can be effectively established on or close to plant roots through rhizosphere competence. Plant development and protection against diseases can be achieved by PGPM root colonisation, and effective root colonisation is thought to require chemotaxis to root exudates (El-Sadoony et al. 2022).

The many species of rhizosphere microorganisms that support plant development are covered. The capacity of PGPM to colonise roots, persist, proliferate on the root surface microhabitats, outcompete native microorganisms, and boost host crop resistance are the factors that classify them (Gamalero et al., 2004). In addition to encouraging plant growth, PGPMs are frequently employed as BCAs to inhibit plant diseases. In agriculture, strains of

plant-associated *Bacillus*, *Pseudomonas*, *Lactobacillus*, and *Actinobacteria* are utilised as BCAs and biofertilizers (Lamont et al. 2017).

Future Perspectives

Biological pest management in sustainable agriculture is an agricultural pest management method that has been shown to be environmentally beneficial. With the help of living microorganisms, this method reduces insect populations in a method that is environmentally friendly, safe, and cautious. When compared to synthetic (chemical) pest management, biological control is a fantastic tool in industrialised nations for achieving safe, affordable, and sustainable pest management. As a result, it benefits consumers and breeders. The antagonistic action of MBCA is seen to be useful alternatives to synthetic fungicides and may also be used to stimulate development and growth of plants for post-harvest objectives. In order to offer answers that support the creation of innovative biocontrol technologies and applications, researchers in the field of MBCA must have novel, and original questions. Additionally, new research avenues targeted at identifying the MBCA-pathogen-plant interaction have been illuminated by bioinformatics, molecular biology, analytical chemistry, and biostatistics (Spadaro and Gullino, 2005).

Conclusion

A multitude of diseases impact a wide range of crops. A sustainable substitute for traditional chemical plant protection is the pest and disease management strategy (PGPM) used in crops. Since they don't impact the environment or public health, these PGPR and PGPF are a safe, efficient, and ecologically friendly method of pest control. Plant health and growth might be enhanced by using PGPR/PGPF, which are antagonistic microorganisms, as biopesticides and biofertilizers. Greenhouse gas emissions and environmental safety may be significantly enhanced by the commercial use of biopesticides and biofertilizers based on PGPR and PGPF.

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CHAPTER 3

Sustainability Issues in the Rice-Wheat Cropping System

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Abstract:

There are issues with sustainability and productivity with the rice-wheat farming system in the Indo-Gangetic Plains of the Indian subcontinent. Although the system has been crucial to ensuring food security, crop growth has slowed down and demand on natural resources has increased recently. Conventional agronomic methods and cultivars are no longer adequate to sustain productivity increases. The issue is made worse by elements including the intensity of labour, water, and energy use, the decline in soil quality, the introduction of new weeds, and climate change. A paradigm shift is required to increase productivity and sustainability; this includes adopting resource-conserving technology, increasing the efficiency with which water and nutrients are used, innovating in residue management, and diversifying crop production. The 4Rs—applying the proper nutrients at the right time, place, rate, and source—can also boost productivity and farm income. It will be essential to address these issues and put suitable measures in place if the rice-wheat farming system in the Indo-Gangetic Plains is to remain sustainable over the long run.

Key words: Rice-Wheat System, Sustainability, Indo-Gangetic Plains

Introduction

Covering an area of 26 million hectares across South Asia and China, the rice-wheat cropping system is one of the greatest agricultural production systems in the world. It involves planting rice and wheat in succession. Due to its production of essential foods, it is indispensable for ensuring food security for almost 20% of the global population. The most significant agricultural areas for this cropping system are found in South Asia's Indo-Gangetic Plains (IGP), which includes portions of Bangladesh, Bhutan, Pakistan, India, and Nepal. About one-third of South Asia's wheat and rice lands and roughly half of Pakistan's and India's total cereal production are produced in the IGP (Banjara et al., 2021). Unfortunately, the high

energy, fertiliser, and water requirements for this system's growth result in higher production costs and more greenhouse gas emissions. There have been concerns expressed about growing environmental footprints and yield stagnation or reduction. The average yield of rice and wheat in this system varies greatly due to variations in soil texture, groundwater levels, water quality, crop output, soil management techniques, and climate variances. However, there are issues with productivity and sustainability with the rice-wheat cropping combination (Dhillon et al., 2021). Natural resources, labour, and energy are getting harder to come by, and yield growth is diminishing. The quality of the soil is declining, new weeds are emerging, and the complexity is increasing due to climate change. The problems include depletion of resources, deterioration of soil health, and environmental harm from burning crop leftovers. India's Rice-Wheat Cropping System has presented challenges and opportunities that have led to serious issues such as diminished soil nutrient reserves, deteriorating soil health, declining groundwater levels, rising production costs, labour shortages, damage to the environment from burning crop residue, increased greenhouse gas emissions, and herbicide-resistant weed species. These difficulties have brought the system's productivity to a halt and jeopardise its long-term viability. Sustainable intensification technologies have been developed to mitigate these problems by lowering labour and irrigation requirements, reducing tillage intensity, and minimising straw burning. To address these problems, a change in strategy is required. To increase productivity and sustainability, recommendations include using resource-saving methods such dry direct planting for rice, zero- or minimal-tillage for wheat, better water and nutrient usage, crop residue management, and crop diversification. In order to overcome the difficulties of the Indo-Gangetic Plains' rice-wheat cropping system, sustainable intensification techniques have been developed. These include lowering the amount of labour and irrigation required, decreasing the depth of ploughing, and minimising the burning of straw. Raising awareness, educating interested parties, and supporting legislation to put flexible techniques into practise locally are the main goals. The difficulties include burning crop residue, which pollutes the environment, dwindling groundwater, unhealthy soil, increased production costs, labour shortages, weeds resistant to herbicides, and climate change vulnerabilities. These issues are addressed by sustainable intensification methods such as laser land levelling, conservation agriculture, zero-tillage, precision nutrient management, and direct sowing of wheat into unplowed soil using shredded rice wastes. Aside, several technical advancements and diversification strategies have not been entirely adopted by farmers because of their expenses, expertise prerequisites, or interoperability with the current framework.

Challenges in the Indo-Gangetic Plains rice-wheat cropping system

The overexploitation of groundwater resources, which has led to a drop in the groundwater table, higher energy costs for pumping water, and deterioration of groundwater quality, is the main factor that has slowed the productivity of the RW system. (ii) Declining soil organic matter and increasing multiple deficiencies of major nutrients (N, P, K, and S).

Traditional Production Practises and Obstacles

In the RWCS, rice is traditionally grown through intense puddling, which is followed by the transplanting of 25–30day old seedlings. Standing water is then left in paddy fields for two weeks, and irrigation is resumed two days after the ponded water evaporates owing to infiltration into the soil (Dhillon et al, 2021). This approach offers a number of benefits, such as reduced oxygen diffusion, which inhibits weed germination, competitive advantages against weeds, assured anaerobic conditions with a neutral soil response, and enhanced availability of minerals like iron. To create a fine seedbed for the wheat crop, tillage operations are repeated after the cultivation of puddled transplanted rice. The primary cause of yield losses in wheat planting is the short time lag between rice harvest and wheat planting, coupled with farmers' inclination to perform excessive tillage in preparation for planting. Planting long-duration coarse rice varieties or medium-duration basmati rice (140 days) may also cause a delay in the planting of wheat. The fields are left fallow for two to three months in between the harvest of wheat and the establishment of rice (Tripathi et al, 2005). Numerous problems have arisen from the traditional method of RWCS cultivation, which are discussed in more detail below.

Health of the Soil

In the standard rice production method, puddling, or tillage in wet conditions, is employed to minimise percolation losses, facilitate seedling transfer, and inhibit weed growth. On the other hand, the ongoing application of this strategy has produced a number of detrimental effects on soil health (Nandan et al., 2021). Subsurface compaction brought on by puddling in coarse- and medium-textured soils affects the cultivation of subsequent highland crops such as wheat by inhibiting root growth and creating aeration problems. Puddled transplanted rice planted after a wheat crop exposes the organic compounds that have been hidden from the air, causing oxidation and structural damage to the soil. Moreover, large aggregates break down as a result of heavy tillage, which lowers crop yields. It has also been demonstrated that continuous RWCS upsets the upper vadose zone's nutritional balance (Bhatt et al, 2021).

Burning rice wastes causes a significant loss of nitrogen, necessitating the use of additional fertilisers by farmers. This raises cultivation expenses and, over time, lowers soil quality. Soil health is negatively impacted by the ongoing RWCS.

Residue Burning

In the RWCS, residue management is a major problem. While wheat residue is not a big deal because it is used in animal husbandry, rice residue (6-8 t ha⁻¹) is not suitable for the dairy industry because of its high silica concentration, low protein content, low digestibility, and poor palatability (Sharma and Dikshit, 2016). Moreover, because of its high carbon:nitrogen ratio, the absorption of fresh rice straw that hasn't broken down immobilises nitrogen in the soil. Because of this, farmers usually turn to burning rice waste, which has several drawbacks. The local population is impacted by crop residue burning-related particle air pollution, as are communities downstream (Lohan et al., 2018). The Indian economy is indirectly impacted by residue burning. Many nations have prohibited flights to Delhi during this period of smog.



Fig 1. Straw burning in farmers' field

Resistance to Herbicides

When a single pesticide was applied consistently to rice to control *Echinochloa spp.*, new hardy weeds like *Leptochloa chinensis*, *Cynodon dactylon*, *Ischaemum rugosum*, *Paspalum distichum*, *Ludwigia hyssopifolia*, and *Phalaris minor* emerged. RWCS monocropping has resulted in increased infestation of wheat because paddy cultivation creates ideal conditions for *P. minor* germination (Sekhawat et al., 2020). Herbicides with similar modes of action were repeatedly applied by farmers, which put a great deal of selection pressure on resistant

P. minor biotypes. Eventually, resistance increased significantly. Three herbicidal modes of action—acetyl-coenzyme A carboxylase (ACCase), acetolactate synthase (ALS), and photosystem II (PS II) inhibitors—have resulted in multiple resistance in *P. minor*. It has been reported that *P. minor* is resistant to isoproturon, clodinafop-propargyl, fenoxaprop-p-ethyl, sulfosulfuron, mesosulfuron-methyl, iodosulfuron-methylsodium, pyroxsulam, and pinoxaden. The weed flora changed when farmers switched to zero-tillage sowing, with broadleaf weeds outnumbering grass weeds (Singh et al, 2021). *Rumex dentatus* developed resistance to metsulfuron-methyl as well as cross-resistance to florasulam, pyroxsulam, iodosulfuron, and triasulfuron after receiving continuous treatment of a herbicide with a comparable mode of action.

Groundwater Depletion

The production of crops, land, and water was impacted by the water table dropping as a result of the RWCS's ongoing use. Moreover, typical rice cultivation in the northwest IGPs results in a considerable loss of irrigation water, especially on coarse-textured soils (Sidhu et al., 2021). Since the 1970s, groundwater levels have been steadily declining in northwest India due to the Green Revolution, causing recurring depletion. One of India's main priorities for resolving the challenging problem of water scarcity is groundwater management. Farmers are being compelled to deepen their tube wells and switch from centrifugal to submersible pumps due to the declining water table, which is driving up production costs and endangering the long-term viability of the current agricultural system (Farmaha et al., 2021).

Economics

Because rice requires a lot of effort to cultivate, both Punjab and Haryana mostly rely on migrant labour. Furthermore, overall profitability and farm revenue have decreased over time due to the growing gap between the total expenses of cultivation and the minimum support prices (which are proxies for market prices) of rice and wheat harvests, even after accounting for the impact of inflation. In contrast, the RWCS continues to be the most popular cropping system in northwest India because of its advantages, which include stable production levels, guaranteed marketing and price (Bhatt et al., 2021). While the RWCS has persisted throughout time, productivity has stagnated since the 1990s, suggesting that the Green Revolution's productivity rate was slowing down (Bhatt et al., 2021). It is necessary to rotate crops instead of depending solely on the RWCS. Crop rotation will yield more economic benefits in addition to resource conservation, particularly with regard to water.

Alternative Production Technologies and Associated Benefits

Over the past three decades, a number of studies have concentrated on finding workable, sustainable, and ecologically acceptable RWCS substitutes (Singh et al., 2020). To enhance declining soil health, lessen groundwater depletion, residue burning, and environmental pollution, as well as to eventually increase farm earnings in a sustainable way, sustainable agriculture-based technology needs to be implemented at the field level (Banjara et al., 2021).

Diversification of Crops

In addition to the RWCS, crop rotations improve soil health, reduce water usage, and increase water yield when used in place of rice. They also increase soil fertility in comparison to the RWCS. In northwest India, water productivity can be raised by growing less water-demanding crops in the summer, such as cotton, maize, pearl millet, or legumes, which will allow for the replenishment of water during the monsoon season (Arora et al, 2020). In northwest India, modifying the cropping calendar, choosing suitable crops and varieties, and altering farming techniques are all methods for controlling water consumption and minimising crop burning. Because of policies that favour these two crops—such as guaranteed output markets, subsidised or free electricity for irrigation, minimum support price (MSP) guarantees, and a dearth of viable and profitable alternative crops for diversification—the government's attempts to replace rice with less water-demanding crops have so far failed (Jat et al., 2020). By gradually raising the MSP, the government has begun to concentrate on pulse crops in recent years. Strong advocacy for alternative policies might persuade farmers in these states to shift from growing rice and wheat to high-value crops like locally grown fruits, vegetables, and flowers.

Crop Residue Management

In the Rice-Wheat cropping system (RWCS), crop residue management is a major concern since loose residue makes tillage difficult and affects wheat sowing. This often forces farmers in northwest India to burn their leftover, which causes a host of problems. Innovative methods of managing residues, such as integrating residues in situ and sowing wheat without tilling using rice residue that has been maintained, have surfaced in response. These methods provide advantages like improved soil health, balanced nutrient levels, decreased pollution, and eventually lower crop costs. The best methods for reducing burning were those for in-situ residue management (Singh et al., 20219). While more sophisticated models, like the Turbo Happy Seeder, allow for quick wheat sowing amid standing rice stubbles after harvest,

innovations such as the zero-tillage drill allow for direct wheat sowing without removing rice residue. Farmers' adoption of various seeding methods and machines for incorporating straw, as well as the new applications of straw for energy and biochar, are being slowed down by the high initial costs and time requirements (Singh et al., 2021). There is still a shortage of a complete set of practises for crops other than rice, such as wheat, potatoes, or vegetables, which calls for more research and development.



Fig 2. Happy Seeder

Technologies for Sustainable Intensification

With its foundations in the ideas of a variety of crop varieties, low tillage, and holding onto crop wastes, conservation agriculture presents itself as a viable means of increasing productivity and maintaining yields in rice-wheat cropping systems (RWCS). A workable substitute for conventional techniques is dry direct-seeded rice (DDSR), which allows for earlier maturation and timely planting of future wheat harvests. But problems still exist, most notably weed invasion and a diverse range of plants. The COVID-19 pandemic-induced labour scarcity sparked a revival in the DDSR area, which was previously declining owing to problems including weed pressure and insufficient rice types. By 2020, it is anticipated that DDSR would cover 200,000–250,000 hectares in Punjab. In order to manage the surface mulching of rice straw and enable no-till wheat seeding, innovations such as the Happy Seeder and rotary disc drill have addressed difficulties in direct wheat planting (Kaushal et al., 2021). Zero tillage, residue retention, and including legumes like *Sesbania* for green manure are all components of sustainable RWCS production (Bhatt et al., 2021).

Improvements like precision irrigation and crop diversity that need less water will help northwest India achieve sustainable agricultural goals within the RWCS framework.

Water-Saving Innovations

Using different water-saving technology is essential for sustainable agricultural practises in the rice-wheat cropping system. Conservation tillage methods, including minimum or no-till farming, can greatly increase soil moisture retention and minimise water evaporation (Sharma et al, 2020). Moreover, the use of laser land levelling reduces water waste caused by uneven surfaces by facilitating effective water distribution throughout fields. When compared to traditional flooding approaches, drip irrigation and alternating wetting and drying (AWD) systems significantly minimise water usage in rice farming (Sapkota et al, 2020). The Rice-Wheat cropping system's water utilisation is further optimised by incorporating drought-resistant crop types that are suited for the area and using moisture sensors to precisely schedule irrigation, which encourages both water conservation and increased crop yields.

Soil Health Improvement

In a rice-wheat cropping system, improving soil health requires a multipronged strategy. By using conservation tillage techniques, such minimum or zero tillage, soil structure, organic matter, and erosion can all be maintained. The management of crop residues is important; keeping rice and wheat remains in the field as mulch improves soil moisture retention and stimulates microbial activity, which in turn promotes nutrient cycling (Parihar et al., 2020). Restoring soil nitrogen levels and reducing disease pressure can be achieved by crop diversification through the addition of legumes or cover crops to the rotation. Furthermore, implementing precision nutrient management strategies guarantees balanced fertilisation, reduces nutrient leaching, and maximises plant absorption. Examples of these strategies include site-specific nutrient administration based on soil testing (Parihar et al., 2020). Lastly, adding trees or shrubs to the cropping system through agroforestry or agroecological techniques can improve soil health even further by promoting biodiversity and adding more organic matter through leaf litter.

Conclusion

The rice and wheat produced by Northwest India's agricultural landscape are essential for feeding the region's expanding population. However, challenges to sustainability such as declining groundwater levels, degradation of the environment, and loss of soil fertility have

beset traditional rice-wheat cropping systems (RWCS). For sustainable crop production, a shift towards conservation agriculture and the adoption of customised techniques is essential. Prioritising the creation of rice varieties with shorter maturation times and higher yields can reduce water loss and residue during harvest. Legumes that grow quickly, such mung beans, can be added to greatly increase total production. A better way to improve sustainable agriculture would be to investigate crops like maize that use less water than rice. Challenges with water inefficiency are addressed by implementing automated irrigation techniques. To achieve agricultural enhancement, it is imperative to scale up sustainable technology like zero-till wheat alongside compatible varieties, raise awareness about enhanced practises, and gather policy support.

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CHAPTER 4

A Comprehensive Overview on Contemporary Approaches in Soil Fertility Management

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Abstract:

A comprehensive overview of contemporary approaches to soil fertility management focuses on three advanced methodologies: Leaf Color Chart (LCC), Diagnostic and Recommendation Integrated System (DRIS), and Soil Test Crop Response Correlation (STCRC). The discussion begins with the Leaf Color Chart, a visual tool that facilitates quick and accessible assessment of nutrient deficiencies in crops. LCC empowers farmers by offering a real-time indication of plant health, enabling timely corrective measures. Next, the Diagnostic and Recommendation Integrated System (DRIS) is explored, emphasizing its role in precision agriculture. DRIS analyzes nutrient balances in plant tissues, providing a nuanced understanding of interrelated nutrient dynamics. This approach enhances the accuracy of nutrient deficiency identification and informs tailored recommendations for optimal fertilizer adjustments. The literature then delves into the Soil Test Crop Response Correlation (STCRC), emphasizing its significance in establishing a direct correlation between soil test results and crop response. STCRC offers a practical link between soil conditions and crop-specific nutrient needs, guiding farmers in targeted fertilization practices. Collectively, these contemporary approaches offer a holistic framework for effective soil fertility management, promoting resource efficiency, environmental sustainability, and improved crop yields. This overview serves as a valuable resource for researchers, agronomists, and policymakers seeking to advance agricultural practices in the pursuit of global food security and sustainable farming.

Keywords: Soil Fertility, Plant Nutrition, Leaf Colour Chart, DRIS, STCRC

Introduction

In the ever-evolving landscape of agriculture, the quest for sustainable and efficient soil fertility management has become paramount. This chapter explores the complex world of

modern methods reshaping the agricultural landscape, with particular emphasis on three key techniques: the Soil Test Crop Response Correlation (STCRC), the Diagnostic and Recommendation Integrated System (DRIS), and the Leaf Color Chart (LCC). The Leaf Color Chart is unique in that it makes it easy and affordable for farmers to quickly and economically evaluate the nutritional health of their plants through visual means (Kumar et al., 2018). Conversely, the DRIS approach utilizes advanced mathematical algorithms to analyze plant nutrient concentrations and provides a thorough framework that helps practitioners achieve the best possible nutrient management (Beaufils, 1973).

Simultaneously, the Soil Test Crop Response Correlation (STCRC) provides farmers with a direct avenue to customize fertilization operations by establishing a vital connection between the results of soil tests and crop responses (Havlin et al., 2014). Together, these strategies highlight a paradigm shift in favor of precision agriculture by placing an emphasis on data-guided decision-making and eco-friendly methods. The importance of these methods in maintaining optimal nutrient balance and sustainable farming practices cannot be stressed, as the agricultural landscape struggles to feed a growing global population while reducing environmental damage. This chapter provides a thorough analysis of these modern methods for managing soil fertility, providing insights into their application and implications for agriculture's future.

Leaf Colour Chart (LCC)

Farmers and researchers may quickly and easily determine the precise nitrogen content of plant leaves by using the Leaf Color Chart. To ascertain the chlorophyll content, which represents the plant's nitrogen condition, LCC uses color-based markers. An easy way to determine whether plants have enough or not nitrogen is to just compare the color of the leaf to a standard color chart (Thind et al., 2012).

Benefits of Leaf Colour Chart in Nutrient Management

- **Cost-effective and Accessible:** For nitrogen estimate, the LCC is a more affordable option than complicated laboratory analyses. Farmers with limited resources can benefit from its accessibility and simplicity, which enables them to make well-informed decisions on nitrogen fertilization.
- **Timely Nitrogen Management:** By offering immediate findings, Leaf Color Chart makes timely nitrogen management possible. Based on the color readings, farmers can

modify their nitrogen application to make sure the plants get the nutrients they need at the proper moment.

- **Monitoring Nitrogen Uptake:** Using the Leaf Color Correlation Coefficient (LCC), regular leaf color monitoring allows for the tracking of plant nitrogen uptake. By using this information, fertilizer application rates and timings can be adjusted to avoid applying too much or too little nitrogen (Kumar et al., 2018).

Limitations and Considerations

While the Leaf Colour Chart offers significant advantages, it is essential to consider its limitations and potential pitfalls for effective nutrient management:

- **Interference from Other Nutrients:** Deficiencies in other nutrients, such as potassium (K) or phosphorus (P), might affect how accurate LCC values are. Diminished leaf color could be a sign of P or K deficiency, which could lead to incorrect conclusions about the amount of nitrogen present. Thus, before depending only on LCC readings, it is imperative to treat any possible deficits of these nutrients.
- **Variability in Crop Types:** Rice crops have been the main focus of LCC development and validation. Although it has also been used to other crops, there may be differences in the relationship between leaf color and nutrient status. More investigation is needed to develop precise LCC recommendations for different types of crops.
- **Calibration and Training:** Proper calibration of the LCC and adequate training of users are critical for obtaining accurate results. Standardized protocols and training programs need to be developed and implemented to ensure consistent and reliable readings across different regions.

Future Prospects

The Leaf Colour Chart has demonstrated its potential as a valuable tool in nutrient management. However, further research and improvements are necessary to enhance its effectiveness and applicability. Some areas for future exploration include:

- **Expansion to Other Crops:** Examining if it is possible to apply the LCC to a wider variety of crops can lead to new opportunities for nutrient management. It is imperative that the relationship between leaf color and nutritional status in various crops be comprehended in order for the LCC to be widely used.

- **Integration with Technology:** Using technology to its full potential can improve the LCC's usability and productivity. Farmers will find the technique easier to use if smartphone applications or portable gadgets that can analyze and interpret leaf color information are developed.
- **Linkage with Soil Testing:** Integrating LCC readings with soil nutrient testing can provide a comprehensive approach to nutrient management. By combining data from both sources, farmers can make informed decisions regarding both nitrogen fertilization and the application of other essential nutrients.

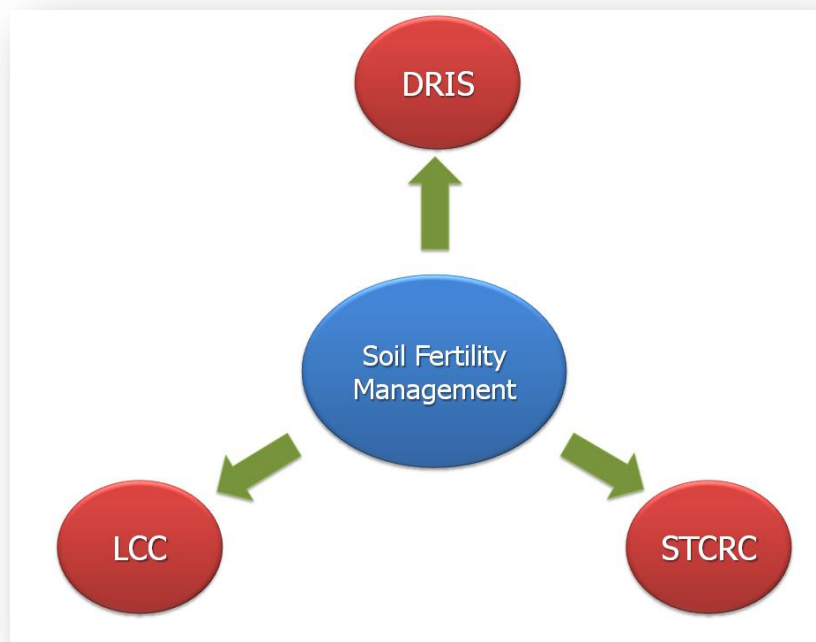


Figure 1: Advances in Soil Fertility Management

Diagnosis and Recommendation Integrated System (DRIS)

DRIS is a technique used in a variety of crops to diagnose nutritional issues. With the added benefit of creating a nutrient surplus or deficiency ranking and measuring the plant nutrient balance, it is regarded as a promising and useful supplementary tool for nutritional diagnostics (Beaufils, 1973). The binary associations between two nutrients are considered by the DRIS technique to be a reliable indicator of the nutritional balance. DRIS indices and graphs are the two formats in which it can be used. While the DRIS indices incorporate the mathematical ranking of the nutrient ratios or products thereof for simple interpretation, the DRIS graphs are only employed in the order of three nutrients and their ratios. The technique has been used on a variety of fruit, ornamental, and horticultural plants, and it has proven to

be just as successful as traditional nutritional diagnostic techniques. There are disagreements about how to calculate the norms and DRIS indices, though, and further study is required to pinpoint and isolate the variables that have a major impact on productivity in various fruit crop management production systems (Srivastava et al., 2022).

DRIS Method in Nutritional Diagnosis

Each ratio function's value is deducted from one index's subtotal and added to another when interpreting nutritional indices using the DRIS approach. The total of the nutritional indices must equal zero; negative findings signify a deficit, whilst high index values indicate an excess of the nutrient in question in relation to the other nutrients. The DRIS indices offer a way to arrange nutrient ratios into understandable expressions for the identification of true nutritional imbalances in plants (Chabi et al., 2023). They are computed using certain formulas.

Application of DRIS

Diagnostic Aspect:

- To determine nutrient concentrations, DRIS uses plant tissue testing.
- Places more emphasis on the interactions between various nutrients than on their individual levels.
- Spots nutritional abnormalities that could be impeding crop productivity.

Nutrient Ratios:

- Examines the proportions of various nutrient concentrations in the tissues of plants.
- Identifies certain dietary excesses or shortfalls in relation to other nutrients.
- Provides a comprehensive perspective on nutrient status as opposed to discrete values.

Recommendation Generation:

- Produces suggestions in response to nutrient imbalances that are found.
- Offers detailed instructions for modifying fertilizer inputs.
- Modifies suggestions to provide a nutrient profile that is more balanced.

Site-Specific and Crop-Specific:

- Makes site-specific nutrition management possible.
- Takes into account the particular requirements of various crops and soil types.
- Improves accuracy while applying nutrients.

Resource Efficiency:

- Promotes appropriate nutrient consumption by crops.

- Reduces the danger of nutritional imbalances and inadequacies.
- Helps agriculture use resources more efficiently.

Sustainability Impact:

- Contributes to sustainable agriculture practices.
- Reduces environmental impacts related with excessive fertilizer use.
- Aligns with the principles of precision agriculture for environmentally conscious farming.

Integration into Overall Nutrient Management:

- DRIS can be incorporated by agronomists and farmers into their nutrient management plans.
- Aids in making decisions for optimizing the use of nutrients.
- Contributes enhancing crop yields while minimizing environmental impact.

Future Research

There are disagreements about how to calculate the norms and DRIS indices, and further study is required to pinpoint and isolate the variables that have a major impact on productivity in various fruit crop management production systems.

Soil Test Crop Response Correlation (STCRC)

To determine the amount of fertilizer and nutrients needed to produce a desired yield, the STCRC approach uses the targeted yield concept. It entails establishing a connection between soil test results, fertilizer applications, and crop yields. Based on the contribution of nutrients from the soil, fertilizers, and organic sources, the targeted yield equation is used to establish the recommended amount of fertilizer. In order to achieve a particular yield, the STCRC technique presupposes a linear relationship between grain production and nutrient uptake (Ramamoorthy et al., 1967).

Role in Agricultural Research

As part of the All India Coordinated Research Project (AICRP), the targeted yield strategy is used to create fertilizer recommendations through the application of the STCRC method. In order to create targeted yield equations that take into account the contribution of nutrients from soil, manures, and fertilizers, it entails conducting field trials in various places. With reference to soil test results, these equations are utilized to produce balanced and quantitative fertilizer doses.

Importance for Food Security and Sustainable Agriculture

By maximizing food grain production from the same area without lowering soil productivity, the STCRC approach is essential for guaranteeing food security. It entails analyzing the soil to identify nutrient imbalances and making suggestions for fertilizer to address these imbalances in accordance with crop needs. This strategy is regarded as one of the most scientific ways to apply nutrients to crops since it makes accurate and significant soil test based fertilizer recommendations and targeted yield equations.

In summary, the Soil Test Crop Response Correlation (STCRC) is a scientifically rigorous method used to establish the correlation between soil nutrient levels, fertilizer inputs, and crop yields. It plays an important role in providing precise and quantitative fertilizer recommendations for achieving targeted crop yields while ensuring sustainable agricultural practices. In order to provide a scientific foundation for improving and maintaining food supply while reducing environmental degradation, the STCRC approach is frequently employed in agricultural research (Kadlag et al., 2016).

Conclusion

The "Contemporary Approaches to Soil Fertility Management" book chapter emphasizes how important cutting-edge techniques like the Soil Test Crop Response Correlation (STCRC), Diagnostic and Recommendation Integrated System (DRIS), and Leaf Color Chart (LCC) are to improving soil fertility management practices. By giving farmers rapid and accurate information regarding nutrient inadequacies, these cutting-edge instruments support precision agriculture by helping them apply fertilizer more efficiently and increase crop yields. A more thorough approach is provided by the combination of LCC, DRIS, and STCRC, which promotes sustainable agriculture practices and enables a more sophisticated knowledge of soil-plant interactions. Adopting these modern methods is essential for assuring effective nutrient management, reducing environmental impact, and increasing agricultural sustainability as the world's food production demands rise. This chapter is an invaluable tool. This chapter serves as a valuable resource for researchers, agronomists, and policymakers seeking to implement cutting-edge strategies in soil fertility management.

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CHAPTER 5

Diversity and Distribution of Honey Bee Species

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Abstract

The sustainability of the ecosystem is greatly depending upon the bees. But their population has been threatened day by day by the various causes including anthropogenic activities as well as natural abnormalities. Hence conservation measures are need of the hour for maintaining the ecosystem sustainability and ultimate the life vulnerability in this universe. Among the various bee pollinators, honey bees are of great importance, as they are being utilised as managed pollinators since for the decades. Besides this, their commercial rearing has been emerged as a new employment generation sector in the rural economy. But nowadays, their rapid population decline causes major threat to the beekeeping industry across the global scenario. But so far as concern, that the locally adapted strains, subspecies and ecotypes of honey bees are less affected from elevated losses than non-native strains. Therefore, conservation of locally adopted strains as genetic resource is essential for future studies. For this purpose a thorough understanding of diversity and distribution pattern of the honey bee species is crucial and simultaneously their adaptation behaviour needs to be analysed for their proper and adequate conservation.

Keywords: honey bee, pollinator, agriculture

Introduction

Bees are hymenopterans, classified as members of the Apoidea superfamily within the Animalia kingdom (Phylum Arthropoda, Class Insecta). A wide variety of bee species can be found in the seven families namely Andrenidae, Apidae, Colletidae, Halictidae, Megachilidae, Melittidae, and Stenotritidae. Broadly, he bees are classified into two groups, the long tongued (L-T) bees and the short tongued (S-T) bees. The elongation of the first two labial palpal segments and the elongation of the galeal blade are the two characteristics that define the long-tongued bees. The short tongued bees, on the other hand, are distinguished by two traits: (i) All labial palpal segments are comparable; and (ii) The galeal blade is typically

short. The long-tongued bees belong to the families Apidae and Megachilidae, whereas the short-tongued bees belong to the remaining 5 families: Andrenidae, Colletidae, Halictidae, Melittidae, and Stenotritidae (Michener 2007).

Till date more than 25,000 species of bees are recognised worldwide. But the number of honey bee species is negligible as compared to overall bee species. The bees that are members of the sole genus *Apis* are referred to as honey bees, or true honey bees and systematically they are members of the tribe Apini of subfamily Apinae, under the family Apidae. They are the truly social or eusocial bees, live in the permanent perennial colonies. The name honey bees came due to their honey storing behaviour, which serves as a complex nutritional sweetener for the colony members to consume during a shortage. Honey bees are humanity's unique gift and people reared them since immortal. The raising of honey bees is known as beekeeping, and it is becoming more and more important in society for both ecological and economic reasons. Honey bee species like *A. cerana* and *A. mellifera* can only be commercially managed by humans, while rest are non-manageable. Apart from honey, they can produce or store different products like beeswax, propolis, bee venom, royal jelly and bee pollen which have valuable market and can provide handsome income. Aside from various bee products, they serve as pollinators, which is their primary ecological function. Pollination is thought to be worth a lot more than the overall value of things produced by bees. Even they regarded as the most effective pollinators because they have particularly designed body parts to take up pollen grains, the ability to operate for extended periods of time, consistent flowers, and temperature tolerance (Free 1964, 1966; McGregor 1976). This chapter provides an overview of variety and distribution range of honey bee species along with their behavioural adaptation.

Types of Honey bee species:

There has been ongoing discussion over the past few decades regarding the number of *Apis* species (Koeniger et al. 2010; Radloff et al. 2011). The number can range from 24 (Maa 1953) at the highest end to 6 or 7 (Engel 1999) at the lowest end, with 10 or 11 species being the most recent (Lo et al. 2010). There are currently eleven known species of *Apis*, and phylogenetic analyses clearly favour grouping them into three separate categories: giant honey bees (*A. dorsata*, *A. laboriosa*, *A. binghami*, *A. breviligula*), dwarf honey bees (*A. florea*, *A. andreniformis*) and cavity-nesting bees (*A. mellifera*, *A. cerana*, *A. koschevnikovi*, *A. nulensis*, *A. nigrocincta*).

Most recently, a new endemic cavity nesting species of honey bee was discovered in the Western Ghats, India and named as Indian black honeybee (*Apis karinjodian*). Its habitat spans the states of Goa, Karnataka, Kerala, and portions of Tamil Nadu, and it extends from the central Western Ghats and Nilgiris to the southern Western Ghats (Shanas et al., 2022).

Table 1: Species of honey bees (type of nest)

Honey bee species having nest with multiple combs (cavity nesting)	Honey bee species having nest with single comb (open nesting)	
	Giant (<i>Megapis</i>) honey bees	Dwarf (<i>Micrapis</i>) honey bees
<i>Apis cerana</i> <i>Apis koschevnikovi</i> <i>Apis mellifera</i> <i>Apis nigrocincta</i> <i>Apis nuluensis</i>	<i>Apis dorsata</i> <i>Apis laboriosa</i> <i>Apis binghami</i> <i>Apis breviligula</i>	<i>Apis florea</i> <i>Apis andreniformis</i>

Giant Honey bees: Subgenus *Megapis*

Giant honey bees are larger in size and can be very ferocious than other honey bees. Due to their fierce nature, they frequently sting their intruders and easily a human being leads to death. They usually build single or a few exposed combs on high tree branches, on cliffs, and sometimes on buildings. In general, traditional method of honey harvesting i.e. by squeezing is practised to collect their honey and the people associated with this known as “honey hunters”. Earlier only one recognised species was there, *Apis dorsata* and many of its subspecies have been described across different locations. But in recent all these subspecies have been recognised as separate species, though controversies are till there regarding this.

The giant honeybee's range is similar to that of the dwarf honeybee, extending from Pakistan (and possibly certain areas of southern Afghanistan) in the west through the Indian subcontinent, Sri Lanka, and Indonesia in the east, with some portions of the Philippines included. Its distribution extends north-south from the southern region of China to Indonesia; it is not found in Australia or New Guinea. Up to 2,000 metres above sea level, *A. dorsata* is found in South China, the Celebes, and Timor but not in Iran or the Arabian Peninsula.

- **Rock bee, *Apis dorsata* F.**

This is one of the biggest honey bees and measuring 16-18 mm. They are mostly found in woodlands and concrete jungles throughout the subcontinent. They construct a single, massive comb (up to one metre square) on the underside of thick, horizontal branches of huge trees, rocks, etc., and have an open nesting style. *A. dorsata* often constructs its nests in the air, between three to twenty-five metres above the ground. Colonies can exist separately or in

groups, a single tall tree may have 10–20 nests and the tree locally called as a “bee tree”. Found in plains as well as hills up to 1600 metres above sea level. They are highly migratory and habitually shift their places. Their general route of migration is hills to plains and vice-versa and roughly seasonal migration occurs 100–200 km distant every year. During winter they migrate from hills to plains and during summer from plains to hills. They are ferocious with having strong swarming or absconding tendency. The mass defensive workers can follow attackers across large distances, up to 100 metres, when they are upset due to their aggressive character (Ramchandra et al. 2012). However, *A. dorsata* workers may fly at night when there is enough moonlight.

The arrangement of comb is comparable to other honeybee species: from top to bottom is honey store, pollen store, worker brood and drone brood. Number of worker cells /10cm comb ranging from 18-19. One colony may yield approximately 40–80 kg of honey per year and the honey is harvested by squeezing (Mishra 1995).

Till now a major portion of Indian honey comes from rock bee. Honey from Sundarban Biosphere Reserve, West Bengal is well renowned in India and the main source of this honey is rock bee. The popular mangroves like Goran (*Ceriops decandra*), Khalsi (*Aegiceras corniculatum*), Keora (*Sonnertia apetala*) etc. are well nectariferous plants and the flowering window of these are well between March to May. The local people popularly known as “Mouleys” used to collect these honey from wild colonies with taking their life risk (Das and Bhuiya, 2020). Though traditional harvesting (by squeezing) is widely followed, but presently due to increasing awareness of bee decline, training programmes are being provided to “Mouleys” for their awareness increasing. As a result, some sort of trained bee hunters prefer to collect only the honey stored portion of the comb, rather than destroy the entire comb. Usually, smoke is applied to calm the bees. However in certain locations experts combine chicken feathers with the smoke to irritate and move out the bees, making easy for honey extraction.

- **The Himalayan Cliff Bee, *Apis laboriosa* Smith**

The largest species of honey bee so far recorded in the world is the cliff bee, measuring up to 3 cm. Lately, *A. laboriosa*, a different species of *Apis*, has been assigned to the enormous honey bees found in Nepal and the Himalayas (Akranakul 1990). The hilly regions of Bhutan, India, and the western Chinese province of Yunnan have also been recognised as home to the bee (Summers 1990; Batra 1995; Ahmad and Roy 2000) They mostly found to nest between 2,500 and 3,500 m of height and forage up to 4100 m and that, in general, they

build nests under overhangs on vertical cliffs (Underwood 1986; Roubik et al. 1985; Sakagami et al. 1980). A little difference in appearance can be found between rock bee and cliff bee, where the later one is not seen in the tropical plains and due to having extensive behavioural adaptations, they are able to construct nests in open at high altitudes; while, the former is common in lower altitudes and plains also, and has a lighter orange brown or tawny body colour. The biology of the cliff bee is mostly unknown, however it is known that it lives in harsh ecological circumstances. Harvesting of honey is very difficult and risky. Mainly the tribal people of these areas (mountainous region) are used to harvest this honey. Harvesters use bamboo poles to hold baskets in place just beneath the combs while they descend rope ladders from the top of the cliff. Once chopped, the comb falls into the basket. Usually, fires are built at the bottom of the cliff to somewhat calm the bees.

There are three varieties of *Apis laboriosa* honey that can be found: autumn honey from any location, spring honey from mid-and lower-altitude, and red honey from higher altitude. In addition to its many relaxing properties, red honey has an intoxicating impact that fades with storage. Because it is valuable, honey hunters typically sell it for a high price, and it is not consumed locally. Red honey is about five times more expensive at wholesale than honey from *A. mellifera* or *A. cerana*. Red honey is highly valued for its alleged therapeutic benefits and euphoric properties.

- ***Apis binghami* Cockerell and *Apis breviligula* Maa**

Some writers have recognised two further morphotypes of gigantic honey bees as whole species: *Apis binghami* Cockerell, found in Celebes east of the Wallace line, and *Apis breviligula* Maa, discovered in the Philippines east of the Meryll line. Both species have elevated ocelli, a uniform black hue, and conspicuous white bands on their abdomens. Maa, 1953 noticed some character differences that Ruttner, 1988 summarised. These distinctions include the fact that *A. breviligula* has a larger abdomen and significantly shorter mouth parts than *A. binghami*, and that nesting aggregations are uncommon for both species—though they are typical for *dorsata* (Morse and Laigo 1969; Starr et al. 1987). Given that these two morphotypes have isolated populations with unique physical characteristics, one could argue that they ought to be treated as distinct species. But since their ranges are allopatric with *A. dorsata*, it's likely that their classification as a species will remain arbitrary.

Dwarf Honey bees: Subgenus *Micrapis*

The dwarf honey bee (Subgenus *Micrapis*) taxa are well represented by *Apis florea* and *Apis andreniformis*, which are called sister bee and having partially sympatric distribution in southern Asia. The distribution ranges varied from tropical to subtropical areas and are found absent from colder climates. Like the giant bee taxa (Subgenus *Megapis*), they also construct a single comb nest in the open place. Hepburn and Radloff (2011) analysed the following traits, which help quickly differentiate *A. florea* from *A. andreniformis*, and they are listed below:

- ✓ The cubital index of worker bees in *A. florea* is approximately three, a substantially lower value than in *A. andreniformis*, where it is approximately six.
- ✓ The hind wing's jugal-vannal ratio in *A. florea* is approximately 75, but in *A. andreniformis*, it is approximately 65.
- ✓ Unlike *A. florea*, *A. andreniformis* has a deeply punctate second abdominal tergite.
- ✓ In *A. florea*, the marginal setae on the hind tibiae are typically completely white, but in *A. andreniformis*, they are sclerotized, dark-brown to blackish, and non-callow individuals.
- ✓ The bifurcated basitarsus of the drones' hind leg in *A. florea* has a significantly longer "thumb" than in *A. andreniformis*.
- ✓ Additionally, the endophallus structure is unique.

- **Red dwarf honey bee or little honey bee, *Apis florea* Fabricius**

This is the smallest species of honey bees so far recognised. Though having of small size it competes well with the other *Apis* species (Koeniger 1976). They possess the similar characteristics behaviour like rock bee, with some exceptions. They are also wild bees and can't be domesticated. They make a single, palm size tiny comb that is attached to the branches of shrubs, hedges, buildings, empty cases, etc. They have an open nesting style. The primary distinction in comb construction between rock bees and little bees is that the former build their combs on the underside of branches, while the latter build their combs around the twigs (Hepburn and Radloff 2011). Found only in plains up to 300 metres above sea level, not in hills. They are generally confined in warm climates and can survive in hot and dry climates with maximum temperatures reaching to 50 °C or more. Their distribution range is similar as giant honey bee: in the west from Oman, Iran and Pakistan through the Indian sub-continent and Sri Lanka, and up to Indonesia to the east, though its primary distribution centre is Southeast Asia. They are mild in temperament with having strong swarming or absconding tendency. Similar to rock bee, they are highly migratory and habitually shift their places

frequently, while attempts for rearing have been found partial success in India (Mishra 1995). They produce very little amount of honey, only about 500 -1000 gm or sometimes more per year per colony (Muttoo 1956; Ruttner 1988). Here also the honey is collected by squeezing (unhygienic way) the comb. The architecture of the comb is akin to other *Apis* species and number of worker cells /10cm comb ranging from 32-36.

Though the data on phenotypic variation of *A. florea* are limiting, three geographic types were identified by Ruttner in 1988: one is found in South India and Sri Lanka, another is from Oman, Iran, and Pakistan, and a third is from Thailand. It is also widely known that India is home to all three of these varieties.

- **Black dwarf honey bee, *Apis andreniformis* Smith**

This one closely resembles to *A. florea*. Both the species are distributed throughout tropical and subtropical Asia and considered as the most plesiomorphic or primitive species alive. Black stripes on the legs, especially on the tibia and on the dorsolateral surface of the basitarsus, are the most important morphological features of the species. The pigmentation of *A. andreniformis* is blackish, while that of *A. florea* is yellowish or reddish. Other distinguishing characteristics include a difference in the respective cubital indexes: *A. andreniformis* has an index of 6.37, and *A. florea* is of 2.86. The proboscis of *A. andreniformis* has a length of 2.80 mm, while that of *A. florea* is 3.27 mm. *A. andreniformis* is generally more defensive than *A. florea* and is known to attack when there are disturbances 3 to 4 m from the hive.

A. andreniformis nests generally in quiet darker forests, where there is 25-30% of normal sunlight and has not been observed in cultivated and inhabited areas. The exposed single combs are built on branches of shrubs and small trees at a height of 1 to 15 m from the ground. The honeycomb typically ranges from 70 to 90 mm in size and the yield potentiality is lesser than that of *A. florea*.

Cavity Nesting Honey Bees:

This category contains honey bees that prefer to make their nests inside of covered spaces rather than outside, such as tree trunks, rock hollows, poles, and other cavities. In addition to their cavity nesting habits, they also construct several parallel combs. Thus, the most significant change or modification in the physiology and behaviour of *Apis* is likely the evolution of cavity nesting over open nesting. These advancements have greatly expanded the

diversity of species and subspecies within *Apis* and enabled it to adapt to a wide range of temperatures.

- **Eastern Honeybee or Indian Bee or Oriental Honeybee, *Apis cerana* F.**

This is a popular species of bee in India, medium in size (11-15 mm) and was the sole managed *Apis* bee until the Italian bee was introduced. In the voids left by fallen trees, in the hollows of rocks, in poles, and in other covered areas, they construct numerous parallel combs similar to *Apis mellifera*. Each colony produces roughly 3-5 kilogramme of honey annually. Here, the honey is extracted from the comb of hived bees using a centrifugal honey extractor, which is a hygienic method. They can be found in Siberia, Northern China, the high alpine region of the Himalayas, and other colder regions of Asia in addition to tropical and subtropical climates (Koeniger, 1976). There are significant differences between the regional populations of tropical and temperate races as a result of the wide spread. There are various subspecies of *Apis cerana*, which can be distinguished by their genetic diversity and living environments. While Engle (1999) acknowledged eight subspecies, Ruttner (1988) only reported four.

- ***Apis koschevnikovi* Buttel-Reepen**

The only places where this species of honey bee has been identified are Sabah, Malaysia, and Indonesian Borneo. Despite having significantly larger individual bees than *Apis cerana*, they are found in the same area as well as the nest construction and size similar with that; hence this one is called as sister bee of *Apis cerana*. The species name was dedicated to Koschevnikov, a pioneer of honeybee morphology in nineteenth century by Buttel-Reepen, who also first described the species. The species was also named as *Apis vechti* by Maa in 1953 for a short period. However, Tingek et al. made the final discovery of it in 1988. Because of their reddish colour when they cluster, they are referred to as red bees locally. This species has been reported as the only host of parasitic *Varroa* species *Varroa rindereri* and seems to be species specific to that (Guzman et al. 1996; De Guzman and Delfinado-Baker 1996).

- **Western Honeybee or European Honeybee, *Apis mellifera* L.**

Apis mellifera, commonly known as the European bee, Western hive bee, or western honey bee, is native to Europe, Africa, and the Middle East. Its strong potential for producing more honey has led to its introduction by human activity to the Americas, Australasia, and much of the rest of the planet. Similar to other nations, it was successfully introduced in India for the first time in 1962 at Nagrota Bagwan (now in Himachal Pradesh; before it was in Punjab

state) (NPCS Board of Consultants & Engineers 2015). Additionally, they are cavity-nesting honey bees, with behaviours akin to those of Indian bees (*Apis cerana*), which construct parallel combs in dimly lit areas (Akranakul 1976; Maa 1953; Otis 1990; Tirgari 1971). Like to *Apis cerana*, they can be domesticated in artificial hives. Based on morphometry, at least 29 subspecies of *A. mellifera* have been described globally (Ruttner 1988; Engel 1999; Sheppard et al. 2003). These are usually classified into four main groups with the help of morphometric and genetic studies as well as analyses of ecological, physiological, and behavioural characteristics: subspecies from all over Africa are included in group A; those from western and northern Europe are included in group M; those from eastern Europe are included in group C; and those from Turkey and the Middle East are included in group O (Ruttner et al. 1978; Ruttner 1988; Garnery et al. 1992; Arias and Sheppard 1996; Franck et al. 2001; Miguel et al. 2011).

Each colony produces 25–40 kg of honey annually. Here also the honey is collected by centrifugal honey extractor (hygienic way) from the comb of hived bees. The architecture of the comb resembles to other *Apis* species and number of worker cells /10cm comb ranging from 17-19.

The many subspecies of *A. mellifera* can be divided by their native continents as follows:

1. European subspecies
2. African subspecies
3. Eastern (Middle East and Asia) subspecies

European subspecies:

Some of the notable subspecies are:

- i. *A. mellifera adami* (Cretan honey bee; in the island of Crete)
- ii. *A. mellifera carnica* (The Carniolan honey bee is found in the northern Balkans, the Eastern Alps, and Slovenia's Carniola region. Its extraordinary softness has made it popular among beekeepers)
- iii. *A. mellifera caucasia* (Central Caucasus is home to the Caucasian honey bee)
- iv. *A. mellifera ceropia* (Southern Greece is home to Greek honey bees)
- v. *A. mellifera cypria* (Cyprus honey bee; located on Cyprus Island)
- vi. *A. mellifera iberiensis* (Iberian Peninsula, comprising Spain and Portugal, is home to the Spanish honey bee)
- vii. *A. mellifera ligustica* (Italian honey bee. Worldwide, they are retained for business purposes. They are extremely mild, unlikely to swarm, and produce a lot of extra

honey. Although some strains are golden, they are primarily light leather-coloured)

- viii. *A. mellifera mellifera* (German honey bee; dark coloured bee, and sometimes known as the European dark honey bee or the German black honey bee)
- ix. *A. mellifera remipes* (Armenian honey bee)

In India, the sub species *Apis mellifera ligustica* or Italian bee had been introduced for commercial beekeeping.

African subspecies:

A. mellifera scutellata, which is primarily found in East Africa and is distributed from Ethiopia to Southern Africa, and *A. mellifera adansonii*, which is primarily found in West Africa, are the two predominant African races of *A. mellifera*. In comparison to the European honey bee, both species are smaller and have more swarms from their nests.

Some of the notable subspecies are:

- i. *A. mellifera adansonii* (West African honey bee)
- ii. *A. mellifera capensis* (Cape honey bee; in Southern tip of Africa. The only bee that can raise a queen from eggs deposited by workers)
- iii. *A. mellifera intermissa* (Tunisian honey bee: found in Tunisia, Morocco, and Libya)
- iv. *A. mellifera lamarckii* (Egyptian honey bee, found only in the Valley of the Nile)
- v. *A. mellifera monticola* (The mountain honey bee of East Africa)
- vi. *A. mellifera scutellata* (Lowland honey bees from East Africa; hybrids called Africanized honey bees)

According to the available research, every subspecies of the African honey bee exhibits distinct behavioural and physical variations (Hepburn and Radloff, 1997), and this heterogeneity has some implications for beekeeping operations. Because of the high number of reported deaths of people and domestic animals, African honey bee races are known for their unexpected defensive attitude, which has created a great deal of dread in the beekeeping community.

Eastern (Middle East and Asia) subspecies:

Some of the notable subspecies are:

- i. *A. mellifera anatoliaca* (Turkey and Iraq's centre region of Anatolia is home to the Anatolian honey bee.)
- ii. *A. mellifera meda* (Iraq's Persian honey bee)

iii. *A. mellifera pomonella* (Tian Shan honey bees are endemic honey bees found in Central Asia's Tian Shan Mountains)

iv. *A. mellifera syriaca* (Syrian honey bee: found in Lebanon, Israel, and Syria)

These subspecies are not appropriate for present-day beekeeping.

- ***Apis nigrocincta* Smith and *Apis nuluensis* Tinget, Koeniger, and Koeniger**

Although *Apis nigrocincta* and *Apis nuluensis* share similar nesting behaviours with *Apis cerana* and *Apis koschevnikovi*, they were only recently identified as separate species from *Apis cerana* (Otis 1997; Tingek et al., 1996). *Apis nigrocincta* inhabits the Philippine islands of Mindanao and Sangihe, while *Apis nuluensis* is found solely in Borneo. The Indonesian islands of Celebes or Sulawesi are also home to *Apis nigrocincta*. In both the species, the parasitic *Varroa* mite species *Varroa underwoodi*, known to cause honeybee disease varroatosis.

Indigenous distribution of honey bees:

Above all the 11 species of described honey bees are not indigenous to all the locations. Among the different continental areas, Asia is believed to be the house of *Apis* species as 10 species out of 11 are indigenous to this. Only the Western honey bee species, *Apis mellifera* is introduced one to this continent. Contrarily, no species of honey bee naturally exists in the Americas, Australia, New Zealand, or Pacific islands; in these regions, the European honey bee has also been established for commercial beekeeping, though stingless bees are native species that have historically been harvested for honey. *Apis mellifera*, the most well-known and extensively researched honey bee, is native to Africa, Europe, and the Middle East, but due to human activity, it has spread across almost every continent except Antarctica.

Table 2: Indigenous distribution of honey bee species

Region	Indigenous honey bee species	Honey bee species introduced
AFRICA	<i>Apis mellifera</i>	<i>Apis florea</i> introduced to Sudan, 1985
ASIA*	<i>Apis andreniformis</i> <i>Apis binghami</i> <i>Apis breviligula</i> <i>Apis cerana</i> <i>Apis dorsata</i> <i>Apis florea</i> <i>Apis laboriosa</i> <i>Apis koschevnikovi</i> <i>Apis nigrocincta</i> <i>Apis nuluensis</i>	<i>Apis mellifera</i>
AUSTRALASIA	No indigenous honeybees	<i>Apis mellifera</i> ,

		<i>Apis cerana</i> has been introduced to Papua New Guinea
EUROPE	<i>Apis mellifera</i>	
MIDDLE EAST	<i>Apis mellifera</i> <i>Apis florea</i>	
THE AMERICAS	No indigenous honeybees	<i>Apis mellifera</i>

* Not all of these species are indigenous to every country of Asia.

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CHAPTER 6

Soil Erosion: Understanding, Conserving and Ensuring Sustainability

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Abstract

Soil erosion, a pervasive environmental issue, results from various natural and anthropogenic factors, causing substantial degradation to arable land worldwide. This abstract explores the causes and effects of soil erosion, highlighting its diverse types such as water, wind, and tillage erosion. Additionally, it delves into the crucial measures and techniques employed for controlling soil erosion, encompassing strategies like terracing, contour plowing, afforestation, and conservation tillage. Estimation of soil loss plays a pivotal role in assessing erosion's impact, employing models and methodologies to quantify the extent of soil degradation. Lastly, the abstract emphasizes the imperative of soil sustainability for future generations, underscoring the necessity of adopting sustainable land management practices to preserve soil quality, fertility, and biodiversity, ensuring a resilient environment for generations to come.

Keywords: Soil Erosion, Soil Conservation, Gully Erosion, USLE

Introduction

Soil erosion, a natural process exacerbated by various human activities, stands as a critical global environmental issue, threatening the very foundation of agricultural productivity and ecosystem stability. This phenomenon, influenced by factors such as wind, water, and human intervention, accelerates soil loss and degradation at an alarming rate. Causes of soil erosion range from deforestation, improper land management, and intensive agricultural practices, to climate change impacts. The effects of soil erosion reverberate across ecological, economic, and social realms, manifesting as reduced soil fertility, compromised water quality, disrupted ecosystems, and increased vulnerability to disasters like floods. Different types of erosion, including sheet erosion, gully erosion, and rill erosion, each manifest unique mechanisms reshaping landscapes and depleting soil resources. Understanding the mechanics of water erosion, a dominant force behind soil degradation, highlights how runoff and sediment transport contribute to soil loss. Agronomical measures for erosion prevention techniques like

contour ploughing, cover cropping, as well as terracing, aim for minimizing soil disturbances and enhance stability. Similarly, engineering methods like the construction of retention walls, check dams, and erosion control structures offer robust interventions to mitigate erosion's adverse impacts. In the context of India, where agriculture remains a cornerstone of the economy, comprehensive measures have been implemented to conserve soil, including watershed management programs, afforestation drives, and promoting sustainable farming practices. Estimating soil loss and assessing its magnitude becomes crucial in devising effective conservation strategies, employing various techniques like modeling, field experiments, and remote sensing. Ultimately, ensuring soil sustainability for future generations necessitates a concerted effort to adopt conservation practices, uphold responsible land management, and prioritize ecological restoration to safeguard this invaluable resource essential for global food security and ecosystem resilience.

Soil Erosion

Soil erosion, driven by wind and water, strips away the top fertile layer, crucial for plant growth, leading to decreased soil fertility. In India, extensive agriculture worsens this issue. Human activities expedite erosion, though natural factors like slope and precipitation play roles. Rain intensity significantly affects erosion, causing engraving grooves in landscapes. Factors like slope angle, vegetation cover, and soil properties influence mass movements. Several studies emphasize soil conservation measures' impact on erosion's soil detachment and transport.

Table 1. Developed mathematical relationships between length of slope (L) and soil erosion (E) (Dar and Meena, 2019)

Researcher's name	Equation
Zing (1940)	$E = f(L^{0.6})$
Musgrave (1947)	$E = f(L^{0.5})$
Wischmeier and Smith (1978)	$E = f(L^{0 \text{ to } 0.9})$
Kornev and Mukhamadullina(1994)	$E = f(L^{0.5})$

Causes of Soil Erosion

Soil erosion causing agents like water, wind, gravity, as well as human actions affect erosion dynamics (Junge et al. 2006). Factors like rainfall, slope, and land use influence erosion rates (Varma et al. 2017). Adaptation strategies, rooted in culture, include crop displacement and

mound planting. Successful traditional methods improve farming and livelihoods (Bukari 2013). Mathematical models linking erosion (E) and slope length (L) are detailed in Table 1.

Effects of Soil Erosion

Soil erosion, a significant environmental issue globally, threatens agricultural sustainability, causing degradation and food production decline (Bukari 2013)

Types of Erosion

It can be broadly divided into two categories::

(a) Geological erosion

It also goes by the name of "normal" or "natural" erosion and describes the simultaneous genesis and loss of soil that preserves the balance between formation and losses. It requires a lot of time.

(b) Accelerated erosion

It is an overabundance of geological erosion brought on by changes in the natural cover or the properties of the soil, either naturally or artificially. The forces of gravity, water, wind, as well as glaciers cause it to happen. The two main factors that accelerate erosion are water and wind. As a result, it is categorized as:

Types of Accelerated Soil Erosion

Wind Erosion

In India, especially in Rajasthan, wind erosion affects arid areas, forming sand dunes and deteriorating soil. Dust emissions impact global biogeochemical cycles, with aridity expected to cover 50% of Earth's surface in a century, threatening food security. Monitoring, aided by remote sensing, improves erosion models and land. Factors fueling wind erosion include soil characteristics, bare smooth surfaces, and powerful winds.

Water Erosion

Rainwater-induced soil movement—erosion—divides into rain, hill, flow, ravine, and current erosion. It detaches fertile soil, impacting nutrients, surfaces, water quality, and agricultural productivity. Factors include physiography, hydrology, land use, vegetation, geology, soil, and climate.

Splash erosion

Raindrop erosion, the initial stage of soil detachment, occurs when raindrops strike the land surface, detaching soil particles that are then carried away by flowing water, commonly

referred to as "splash erosion." The impact of raindrops can propel soil particles up to 50–75 cm into the air, with gravity directing more soil material toward the slope. The stability of soil aggregates during raindrop erosion hinges on rainfall properties such as drop size, kinetic energy, and intensity. While raindrop impact can directly dislodge soil clusters, it remains debated whether the impact pressure alone can destroy these clusters outright. Moreover, soil properties like clay content, organic carbon, water content, and cation exchange capacity significantly influence raindrop erosion.

Sheet erosion

Sheet erosion involves the detachment of a uniform layer of soil by rainfall and runoff, often inconspicuous but steadily depleting the upper fertile soil layer.

Rill erosion

Rill erosion, characterized by the formation of small branched canals, acts as an intermediate phase between sheet erosion and the advanced stage known as gully erosion.

Gully erosion

Gully erosion, the culmination of water erosion, results in the formation of ravines due to unattended furrows, influenced by factors like improper land practices and topography.

Stream bank erosion

It arises from stream or waterway movements, necessitates careful consideration of hydraulic principles for safeguarding neighboring lands and infrastructure. These erosional processes, each distinct in its manifestation, underscore the intricate relationship between natural phenomena and human activities in reshaping landscapes and degrading soil integrity.

Mechanics of Water Erosion

Water erosion, influenced by human activities, intensifies due to vegetation removal and mismanagement of land. Rainstorms expose soil surfaces, leading to erosion, influenced by rain intensity, soil nature, slope, and other factors.

Agronomical Measures of Erosion Control

Agronomical erosion control methods involve using vegetation to control soil erosion on gentle slopes (<2%), offering lasting and cost-effective measures. These methods enhance infiltration and cut down on surface runoff. The following agronomic practices are frequently employed to reduce water erosion:

Contour cultivation

The practice of cultivation on contour involves executing all agricultural operations along the contour of the land, reducing velocity of surface flow and delaying soil removal by erosion. Instead of going up and down the slope to sow crops, contour trimming effectively minimizes erosion, particularly on slopes ranging from 2% to 10%. This method utilizes crops like corn, sorghum, and millets to protect the upper soil, aiding water infiltration and runoff reduction.

Strip cropping

It is an intensive agricultural practice, involves cultivating different crops in alternating strips along contour, controlling surface runoff and promoting rainwater preservation. It combines practices like boundary farming, cover crops, conservative tillage, and crop rotation to enhance soil moisture retention and reduce erosion rates. Different kinds of strip cropping, including Contour strip, field strip, buffer strip and wind strip cropping, are employed based on topography and erosion concerns, emphasizing erosion control methods aligned with slope and wind directions (Suresh, 2012)

Cropping System

Crop rotation enhances soil fertility, prevents erosion, and diversifies nutrient demands among different crops, mitigating soil exhaustion. Legumes in rotations aid fertility and reduce emissions. Agricultural practices, fertilization techniques, and timing significantly impact nitrogen emissions like N_2O . Nitrification and denitrification from nitrogen sources influence N_2O production, affected by oxygen levels and fertilizer application. Spring fertilizer applications in the US Corn Belt during wet seasons promote denitrification and significant N_2O emissions.

Tillage Practices

Tillage Practices manipulate soil for optimal crop growth, maintaining infiltration, controlling weeds, and preventing erosion. Over-tillage damages soil structure, impacting N_2O emissions. Conservation agriculture, defined by minimal soil disturbance, specific land cover, and crop rotation, helps protect against erosion. Conservative tillage practices like minimum, strip, and mulch tillage sustain yields by reducing costs and improving soil conditions for germination and water absorption. Vertical mulching aids in drainage, controlling erosion and soil temperature.

Engineering Methods of Erosion Control

Terracing

Terracing, a practice suited for steep slopes, divides them into sections, curbing runoff and erosion while enhancing soil depth and water retention. In India, it includes Bench terraces for various crops based on rainfall and soil permeability, offering diverse functions and

designs. Broad base terraces, categorized as graded and level types, are also employed based on land slope and rainfall conditions.

Bunding

Bunding is an effective technique in controlling runoff and curbing ground erosion. Bunds, embankment structures, aim to decrease the pitch length, reducing soil erosion, particularly appropriate for areas with gradients ranging from 2 to 10%. However, they may not be appropriate for black soils due to crack development. In sub-Saharan Africa, inland valleys, encompassing over 38% of wetlands, are extensively used for rainy rice cultivation. Despite this, the performance of such crops remains down due to different biophysical factors and insufficient crop management practices. Understanding the topographic sequence's correlation with natural resources like water and soil in these areas is crucial for enhancing agricultural practices. Notably, West Africa's rice cultivation in lowlands differs significantly from Asia due to the absence of water control systems. Research has shown bunds' efficacy, enhancing harvest productivity by 30–100% in Tanzania. To optimize their effectiveness, bunds are recommended at a height of 0.3 meters for plots of a maximum of 0.10 hectares, with soil classification determining their appropriate depth for construction. Bunds are further classified as contour bunds, constructed along contours, and graded bunds, specifically recommended for areas experiencing high annual precipitation. Contour bunds are suitable for slopes around 6% and regions with rainfall <600 mm annually, while graded bunds are utilized where rainfall exceeds 600 mm, within specific slope limits.

Grassed Waterways

Grassed waterways are highlighted as natural or artificial watercourses lined with grasses that resist erosion, strategically designed to redirect surface water away from agricultural lands. These waterways serve various purposes, including diverting runoff, transporting sewage, or functioning as emergency discharge channels. Grassed waterways, constructed along the land's slope, aid in erosion prevention and moisture conservation. Singh and Kanwar (1991) have provided essential values of velocity that was non-erosive for the secure design of such waterways.

Check Dam

Check dam is nothing but small barriers constructed using stones or sandbags, intended to reduce flow velocity and subsequent erosion. They serve both permanent and temporary purposes, enhancing soil moisture and facilitating aquifer recharge. Check dams effectively mitigate the risk of land submergence during flash floods (Suresh, 2012).

Measures to Conserve Soil in India

In India, soil erosion reduction strategies encompass the establishment of contour lines, forestry regulations, grazing control, crop rotation, and mixed farming. Central government initiatives during various Five-Year Plans have focused on soil conservation. These include the division of land and water Conservation, addressing erosion issues in agriculture. Specific projects include regenerating alkaline soils (7th FYP), water basin development in northeastern states (8th FYP), River Valley and flood management (9th FYP) (Anonymous 1979), and ravine recovery programs in states like Rajasthan and Madhya Pradesh. Despite these efforts, educating rural communities about soil conservation remains crucial due to inadequate implementation of these initiatives.

Soil Loss Estimation

The loss of soil due to various erosion types precipitates a parallel loss of essential nutrients. Accurate estimation of soil loss remains crucial in understanding the impact of diverse land management measures. Methods to quantify soil loss involve intricate processes such as employing flow diagrams for sheet erosion evaluation and utilizing H-fume and Coshocton samplers combinely to estimate runoff and sediment in agricultural water basins. While numerous equations were proposed by scientists for soil loss estimation, limitations existed until the formulation of the Universal Soil Loss Equation (USLE) by Wischmeir and Smith (1965). This model, accounting for factors causing erosion, predicts long-term yearly erosion rates in slopy fields, incorporating precipitation, soil type, topography, and cultivation practices. The USLE's empirical nature, based on extensive runoff and ground loss data, facilitated its widespread application, defining erosion dynamics within the context of a standard unit plot. However, the model's focus on average erosion rates over extended periods omits consideration of individual events' contributions and the influence of rare events on long-term averages. The Universal Soil Loss Equation (USLE) is

$$A = R.K.L.S.C.P$$

where

A= Average annual soil loss in $t \text{ acre}^{-1}$ (tons per acre)

R= Rainfall erosivity index

K= Soil erodibility factor

LS= Topographic factor; L is for slope length and S is for slope

C= Cropping factor

P= Conservation practice factor

The USLE's structure primarily includes factors like soil erodibility, rainfall erosivity, , topographic influence, cropping, and conservation measures and practices, enabling the estimation of average annual soil loss. Despite its efficacy, the USLE does not directly incorporate runoff or sediment concentration for individual events. Subsequent refinements like RUSLE and MUSLE aimed to address these limitations. Studies applying these equations, such as one conducted by Kumar et al. in the Khimbar micro-watershed, estimated substantial soil loss, showcasing the equations' utility in quantifying erosion rates and aiding land management decisions.

Soil Sustainability for Future Generation

Sustainability is the ability to meet needs now without endangering future generations. For soil sustainability, soil erosion control is essential; degraded soil compromises yield of crops. Healthy soil is pivotal for sustainable agriculture. Protecting soil through various means is crucial, including using biological fertilizers, incorporating biochar, and promoting organic farming. Organic farming sustains healthy soils, fostering nutrient-rich environments for long-term productivity. Achieving sustainable agriculture necessitates soil sustainability, employing conservation methods and improved cultivation practices.

Conclusions and Future Perspective

Reductionist research yields vital soil insights, but alone, it can't address complex issues like weather changes, landscapes, and cultivation systems. Incorporating varied factors and novel approaches enhances spatial inference, guiding research and aiding environmental solutions. Nature's offerings, often overlooked, hold potential in addressing society's environmental challenges if researchers prioritize understanding and applying its laws.

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CHAPTER 7

Scientific advances in heavy metal pollution in the soil environment

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Abstract

Heavy metal pollution in the soil environment refers to the presence of elevated levels of metallic elements that can be destructive to both environment and human wellbeing. Common heavy metals of concern include lead, cadmium, mercury, arsenic, and chromium, among others. Human actions such as engineering and industries, mining, agriculture, and inappropriate waste dumping contribute appreciably to the contamination of soil with these heavy metals. Once released into the soil, heavy metals can persist for long periods and build up in the soil, posing a risk to plants, animals, and humans. Plants can absorb these metals, leading to reduced crop yields and potential health risks for those consuming contaminated produce. Additionally, soil-dwelling organisms may be adversely affected, disrupting the overall ecosystem balance. Contaminated soil can also serve as a source of water pollution, as rainwater or irrigation can wash the metals into nearby water bodies, further spreading the environmental impact. The health risks associated with heavy metal exposure include developmental issues, organ damage, and various diseases in humans. Efforts to address heavy metal pollution in soil involve remediation techniques such as phytoremediation (using plants to absorb and accumulate metals), soil washing, and microbial remediation. Strict regulations and sustainable practices in industries and agriculture are essential to prevent further contamination and protect both the environment and public health.

Keywords: soil, pollution, heavy metal, health risk

Introduction

The term "pollution" has become a common occurrence in our ordinary and day-to-day lives. Pollution occurs when many sources of pollutants contaminate our natural environment, leading to changes that negatively impact our daily lives. These contaminants, which can be found as waste in a variety of forms, are thus the main components of pollution. This pollution causes an imbalance in the ecology and environment, which is our most important

resource. Because it collects and sometimes concentrates contaminants, soil serves as a natural absorber for them. Despite the fact that most pollutants are the result of human action, some may also come from the environment. While soil pollution is defined as the occurrence of a chemical as well as any substance that is in the wrong place, present at elevated than normal concentration, and/or has detrimental effect on any untargeted organism, soil contamination relates to the existence of a chemical or any substance in soil at an amount that is greater than would be expected naturally but is not directly causing harm (Sengupta et al., 2021).

Causes of soil pollution

Both natural and man-made factors can contribute to soil pollution (Fig 1). Natural sources of pollutants may include elements of minerals found naturally in soil that, at larger concentrations, can be hazardous to people, animals, or plants. Because of centuries of human activity, undesired materials have been introduced into the natural system, and the majority of these pollutants are to blame for global soil contamination. According to studies, 5 to 6 million hectares of cultivable land globally are gone astray permanently every year as a result of soil degradation and other factors (Havugimana et al., 2017).

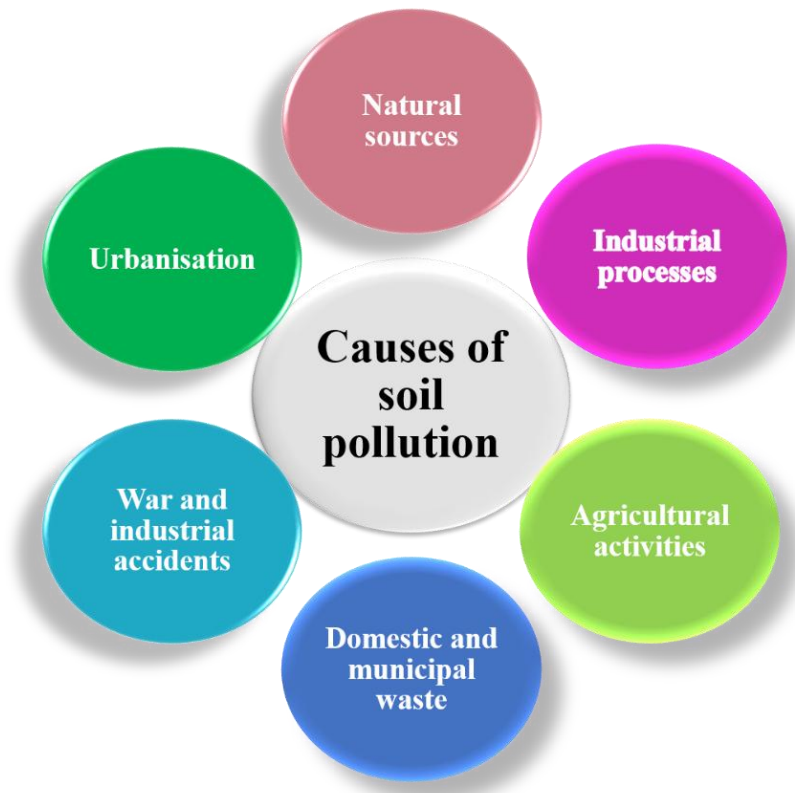


Fig 1. Schematic depiction of causes of soil pollution

Effects of Soil Pollution on the Environment

A broad range of pollutants can be found in soil, including heavy metals, pesticides, herbicides, petroleum products, and industrial chemicals. Heavy metals, such as lead, cadmium, and mercury, are particularly problematic due to their long existence in the environment and their potential to cause serious health effects. Pesticides and herbicides, commonly used in agriculture, can also pollute the soil and have detrimental effects on both human wellbeing as well as the environment. The consequences of soil pollution are far-reaching. Contaminants seep into the soil, affecting its nutrient composition and disrupting the delicate balance of microorganisms that contribute to plant growth. This, in turn, affects crop yields and can threaten food security. Moreover, pollutants in the soil can leach into groundwater, polluting drinking water sources and endangering human health. Additionally, soil pollution negatively impacts biodiversity by harming beneficial organisms and disrupting ecosystems. Soil pollution poses significant risks to human health. When pollutants in the soil enter the food chain, they can accumulate in crops and ultimately be consumed by humans. This can lead to the ingestion of toxic substances, causing a range of health issues, including organ damage, developmental disorders, and increased cancer risk. Children and pregnant women are mostly susceptible to the harmful effects of soil pollution (Saha et al., 2017).

Heavy metals in the environment

Heavy metal pollution is a result of various human activities, including industrial processes, mining, agriculture, and improper waste management. These activities release heavy metals into the environment, where they accumulate and create a significant hazard to ecosystems and human health. Industrial processes, such as metal smelting, coal combustion, and manufacturing, release heavy metals into the air as well as water bodies. These metals can then settle on the ground or be carried by wind and rain, contaminating soil and water sources. Mining activities, especially in areas with rich mineral deposits, can cause heavy metals to be released into the environment where they can contaminate water bodies and soil. Agricultural practices, such as the application of fertilizers and pesticides, can also add to heavy metal pollution. These substances contain heavy metals, which can seep into the soil and water sources, contaminating crops and entering the food chain. Improper waste management, including the disposal of electronic waste, batteries, and industrial waste, can further exacerbate the problem by releasing heavy metals into the environment (Long et al., 2021).



Fig 2: Arsenocosis (Skin lesions) caused by arsenic

Heavy metals, such as lead, mercury, cadmium, and arsenic, are particularly toxic. These metals have various sources and can come into the environment through natural as well as human activities. Lead is a common heavy metal pollutant that can be found in old paint, contaminated soil, and industrial emissions. It may penetrate the body of a human by skin contact, ingestion, or inhalation. Lead exposure can have severe health effects, especially in children, including developmental delays, impaired cognitive function, and damage to the nervous system. Mercury, another dangerous heavy metal, is released into the environment through industrial processes, coal combustion, and the incineration of waste. It accumulates in fish and seafood, making it a significant concern for human consumption. Mercury exposure can lead to neurological disorders, impaired fetal development, and kidney damage. Cadmium, often found in batteries, pigments, and fertilizers, can contaminate soil and water sources. It accumulates in the liver and kidneys and can cause kidney damage, respiratory problems, and an increased risk of cancer. Arsenic, a naturally occurring heavy metal, can contaminate groundwater and pose a serious health risk when consumed through drinking water or contaminated food. It is known to cause skin lesions (Fig 2), cardiovascular diseases, and various types of cancer (Sengupta et al. 2023; Das et al., 2023).

Impact of Heavy Metal Pollution on Ecosystems

Heavy metal pollution has devastating effects on ecosystems, disrupting the delicate balance of nature. Aquatic ecosystems are particularly vulnerable to heavy metal contamination, as these metals can build up in water bodies and affect various organisms, from plankton to fish. Fish are highly sensitive to heavy metals, as they can accumulate in their tissues over time. This not only affects the health of the fish but also poses a significant risk to those who consume them. Birds and other wildlife species that rely on fish as a food source can also be indirectly affected by heavy metal pollution (Meena et al., 2018).

In terrestrial ecosystems, heavy metal pollution can contaminate soil, affecting plant growth and reducing biodiversity. Heavy metals can be absorbed by plants from the soil, and when consumed by herbivores, these metals can be transferred up the food chain, ultimately affecting predators as well. The entire ecosystem may be affected in a cascade manner by this disturbance in the food chain. Numerous creatures' ability to reproduce can be impacted by heavy metal pollution, which can result in population decreases and ecological imbalances. Additionally, the addition and build up of heavy metals in the environment can persist for long periods, making the cleanup and restoration of contaminated areas a challenging task.

Water bodies, such as rivers, lakes, and oceans, are highly susceptible to heavy metal pollution. Industrial discharges, sewage, and runoff from agricultural fields are major sources of heavy metals in water. Once released into water bodies, heavy metals can persist for a long time, as they do not break down easily. They can accumulate in sediments, affecting the health of bottom-dwelling organisms and disrupting the balance of aquatic ecosystems. The contamination of water sources with heavy metals can also have severe consequences for human health, as these metals can enter the drinking water supply. The impact of heavy metal pollution in water bodies extends beyond the immediate area of contamination. Downstream communities can also be affected, as heavy metals can travel long distances through waterways, ultimately reaching the ocean and impacting marine life.

Soil contamination with heavy metals poses a significant threat to agriculture and food security. Heavy metals can enter the soil through the deposition of airborne particles, the use of contaminated irrigation water, and the application of fertilizers and pesticides containing these metals. Plants can absorb heavy metals once they are in the soil, leading to reduced crop yields and quality. This not only affects the livelihoods of farmers but also poses a risk to consumers, as these metals can accumulate in edible parts of plants. Contaminated soil can also impact soil microorganisms and beneficial insects, affecting the overall health of the ecosystem. To mitigate the dangers of soil contamination by heavy metals, proper soil

management practices, such as crop rotation, phytoremediation, and the use of organic fertilizers, should be implemented (Bhattacharyya and Sengupta, 2020). Regular soil testing and monitoring are also crucial to identify and handle the contamination of heavy metals in agricultural regions.

Heavy metals can also be present in the air we breathe, posing a threat to human health and air quality. Industrial emissions, vehicular exhaust, and the burning of fossil fuels are major sources of heavy metals in the atmosphere. Once released into the air, heavy metals can be transported over long distances, affecting both urban and rural areas. Inhalation of heavy metal particles can lead to respiratory problems, such as asthma and bronchitis. Long-term contact to airborne heavy metals can also increase the peril of lung cancer and other respiratory diseases. The impact of heavy metal pollution on air quality extends beyond human health. These metals can settle on vegetation, impairing photosynthesis and reducing plant growth. This, in turn, can have cascading effects on ecosystems, as plants are essential to the survival of a variety of organisms.

Methods of Detecting and Measuring Heavy Metal Pollution

Identifying and quantifying contamination from heavy metals is essential for assessing the extent of contamination and implementing appropriate remediation strategies. Various methods and techniques are utilized in the subject of environmental monitoring to determine the presence and concentration of heavy metals in different environmental compartments (Kurup et al., 2017). Laboratory analysis is commonly used to measure heavy metal levels in water, soil, and air samples. Techniques such as atomic absorption spectrophotometry, inductively coupled plasma mass spectrometry, and X-ray fluorescence spectroscopy are employed to quantify the concentration of specific heavy metals. In addition to laboratory analysis, field-based techniques, such as portable X-ray fluorescence analyzers and field kits, are available for rapid screening of heavy metal contamination. These tools provide quick results, allowing for on-site assessment and decision-making. Remote sensing technologies, including satellite imagery and aerial surveys, can also be utilized to identify areas of potential heavy metal pollution. These methods provide a broader perspective, allowing for the monitoring of large-scale environmental changes over time.

Recent scientific advances in the study of heavy metal pollution in the soil environment have provided valuable insights into the mechanisms of contamination, as well as innovative approaches for detection and remediation. Advanced diagnostic techniques, such as X-ray fluorescence spectroscopy, atomic absorption spectroscopy, and inductively coupled plasma

mass spectrometry, enable more accurate and sensitive measurements of heavy metal concentrations in soil. Molecular biology tools, including DNA-based technologies, help in understanding the microbial communities involved in metal transformations and the development of bioindicators for assessing soil health. Nanotechnology has emerged as a promising field, offering nanomaterials for efficient remediation of heavy metals in contaminated soils. Additionally, remote sensing and geospatial technologies aid in mapping and monitoring the extent of heavy metal pollution over large areas. The intricate dynamics of heavy metal pollution in soil are better understood thanks to scientific developments, which also offer practical solutions for environmentally friendly soil management.

Steps to Reduce and Prevent Heavy Metal Pollution

A multifaceted strategy including industry practises, governmental restrictions, and individual initiatives is needed to address heavy metal contamination (Shaheen et al., 2020). Here are some steps that can be taken to reduce and prevent heavy metal pollution:

1. **Regulations and Policies:** Governments should establish and enforce strict regulations on the environmental discharge of heavy metals. These regulations should cover industrial emissions, waste management practices, and agricultural activities.
2. **Waste Management:** Appropriate methods for managing waste, such as recycling and securely disposing of electronic debris, batteries, and industrial waste, are essential to prevent heavy metal pollution.
3. **Clean Technologies:** Industries should adopt cleaner technologies and processes that minimize the discharge of heavy metals. This includes the use of pollution control devices and the implementation of best practices in manufacturing and waste management.
4. **Education and Awareness:** It is imperative to raise public understanding of the risks posed by heavy metal contamination. Campaigns for education can aid people in understanding the sources of heavy metal pollution and take necessary steps to reduce their exposure.
5. **Monitoring and Testing:** Regular monitoring and testing of air, water, and soil for heavy metal contamination are essential to identify problem areas and take appropriate remedial measures.
6. **Phytoremediation:** Utilising plants to extract heavy metals from contaminated soil and water is known as phytoremediation, and it can be a successful and long-lasting method of cleaning up polluted areas.

7. **Collaboration and Research:** Collaboration between governments, industries, and research institutions is essential to develop innovative solutions and technologies to address heavy metal pollution effectively.

Conclusion and Future Prospects for Tackling Heavy Metal Pollution

The primary sources of soil contamination are a variety of human activities and experiments. Industrial wastes, including chemicals and hazardous gases like pesticides, fertilizers, and insecticides used in agriculture are the most frequent sources of soil pollution. Despite the fact that soil is a non-renewable natural resource, humans have increasingly used it as a pollutant sink since their inception. Ignorance about soil management is one of the factors leading to the build up of pollutants in soil. It has been associated to negative impacts on soil microorganisms. There is a serious risk to ecosystems, human health, and the environment from heavy metal pollution. The consequences of heavy metal contamination can be far-reaching, affecting everything from air and water quality to agricultural productivity and biodiversity. In order to effectively combat heavy metal pollution, governments, businesses, and individuals must work together. We can lessen the amount of heavy metals released into the environment and stop additional contamination by enacting stringent legislation, introducing cleaner technologies, and encouraging sustainable practices. Accurate testing and assessment methods are essential in identifying and monitoring soil pollution. Various techniques, such as soil sampling and laboratory analysis, allow for the detection and quantification of pollutants. Additionally, advanced technologies, including remote sensing and geospatial analysis, can provide valuable insights into soil pollution patterns and help prioritize areas for remediation. Furthermore, further investigation and creativity into the problem of heavy metal contamination may result in the creation of environmentally friendly substitute materials, better remediation plans, and monitoring methods.

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CHAPTER 8

Enhancing the Production of Secondary Metabolites in Medicinal Plants via Hairy Root Culture Using *Agrobacterium rhizogenes*

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Abstract

When it comes to developing new drugs, plants are a great source for discovering novel compounds with therapeutic value. Numerous unique plant-derived compounds are employed in numerous significant applications. Economically, secondary metabolites are valuable as flavorings, insecticides, food additives, flavors, and dyes. Plants generate a wide range of secondary metabolites, which are crucial for environmental adaptation and a significant source of active medicinal ingredients. One of the burgeoning areas of biotechnology to explore and improve the production of secondary metabolites is the potential to modify the synthesis of bioactive plant metabolites via tissue culture technologies. Due to biotic and environmental conditions, this augmentation through field cultivation has many flaws, including low and variable yield and delayed growth. In order to overcome the limitations of seasonal, climate, and environmental fluctuations, hairy root culture has been created as a more effective alternative biotechnological technique for secondary metabolite production. Due to their quick development in conditions devoid of phytohormones, biochemical and genetic stability, and hairy roots created via genetic transformation, in vitro hairy roots have shown to be an effective tool for the synthesis of greater quantities of flavonoids. This chapter's main objective is to evaluate studies on rhizogenesis in various plants, specifically utilizing *Agrobacterium rhizogenes* to increase secondary metabolites.

Keywords: Hairy root cultures; *Agrobacterium rhizogenes*; medicinal plants; secondary metabolites

Introduction

Secondary metabolites from plants are unique sources of flavorings, food additives, medicines, and industrially significant biochemicals. When plants are under stress, they frequently accumulate these metabolites, which might include different elicitors or signal molecules. Plants use secondary metabolites to help them adapt to their surroundings and get

through stressful situations. Humidity, temperature, light intensity, water availability, mineral content, and CO₂ all have an impact on a plant's development and the synthesis of secondary metabolites. Modern technology's main benefit is that it may offer a consistent and dependable supply of plant medicines and be used to the large-scale cultivation of plant cells so that these metabolites can be isolated. Plant tissue and cell cultures have a significant deal of potential for the on-demand, regulated synthesis of valuable secondary metabolites. The commercial objective of the plant cell-based bioprocess for the generation of the majority of secondary metabolites cannot be realized by the existing yield and productivity. Recent developments, fresh perspectives, and promising prospects in plant cell-based techniques are being closely scrutinized in order to push the envelope. It is necessary to think about ways to increase the synthesis of secondary metabolites. The absence of certain precursors limits the output of the necessary metabolites. The buildup of chemicals may be enhanced via biotransformation employing genetic engineering, metabolic engineering, and an external source of biosynthetic precursors.

Agrobacterium rhizogenes, or *Rhizobium rhizogenes* as it was recently amended, is the causative agent of hairy root disease in a large variety of dicotyledonous plants and several gymnosperms. Gram-negative soil bacteria *A. rhizogenes* infects plants, causing adventitious roots, sometimes known as "hairy roots," to sprout from the infected area (Fig. 1). This phenomenon is caused by the entry into the plant genome of a specific DNA region known as transfer DNA (T-DNA), which consists of the loci spanning the TR and TL sections of the bacterium's root-inducing (Ri) plasmid (Su and Lee 2007). Although the roles of various genes on the T-DNA have not yet been determined, the basic molecular mechanism of T-DNA trimming from the Ri plasmid, transport to plant cells, and its incorporation into the plant genome is understood. Without the addition of phytohormones, the hairy roots are aseptically cultivated in vitro. The plant's incompatibility with a specific technique presented a challenge for the in vitro production of secondary metabolites from plants. Due to the unique genetic makeup of each plant, applied technique results are not always progressive. To succeed, it needs to be tuned using various circumstances and bacterial strains. An overview of the use of elicitors and hairy root cultures mediated by *A. rhizogenes* for the synthesis of secondary metabolites in medicinal plants is the aim of this study. There are several elicitors that have been shown to increase the plant's production of secondary metabolites.

Patterns of increased secondary metabolite production in plants

To facilitate the multiplication and extraction of secondary metabolites, it is possible to routinely establish plant cell and tissue cultures under sterile conditions from explants, such as plant leaves, stems, roots, and meristems? Enhancement in the production of secondary metabolites can be achieved through strain improvement, high-producing cell line selection techniques, and medium optimization. It has been known practically since the beginning of in vitro technology that plant cell, tissue, and organ cultures are capable of producing and accumulating many of the same valuable chemical compounds as the parent plant in nature. Refocusing on in vitro plant compounds as potential factories for secondary phytochemical products, the strong and increasing demand for natural and renewable products in today's market has allowed for new research investigating secondary product expression in vitro (Hussain et al. 2012).

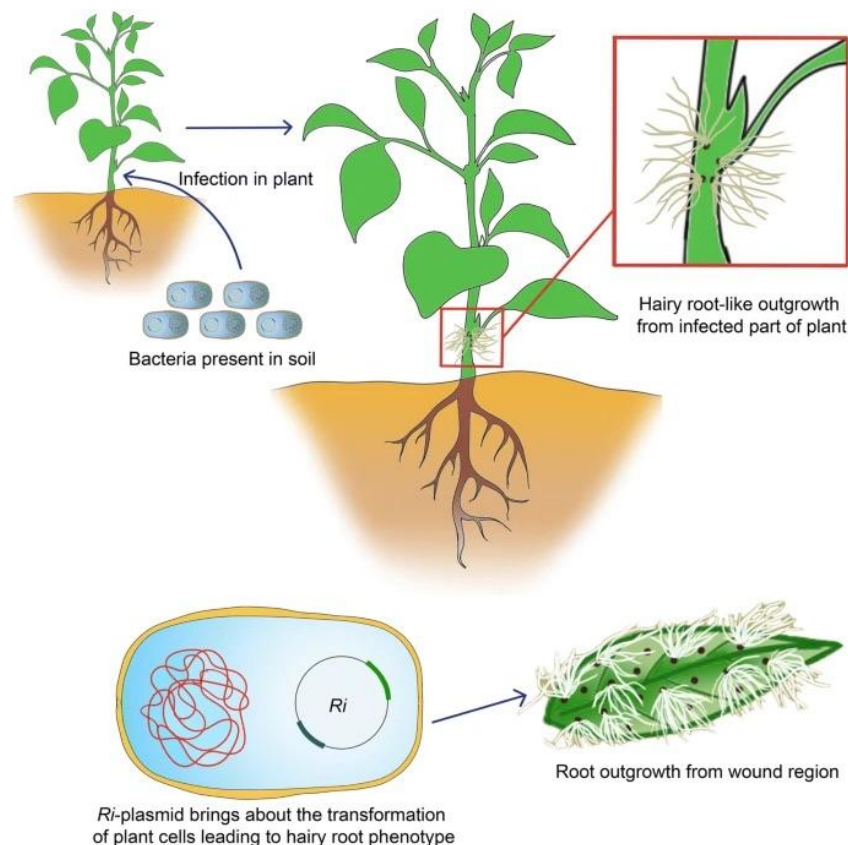


Fig. 1: Diagrammatic representation of hairy root induction in plants by the bacteria *Agrobacterium rhizogenes* under natural conditions. [Gantait and Mukherjee (2021)]

Precursor addition for increased synthesis of secondary metabolites

Several kinds of precursors can be added to hairy root cultures to increase the production of secondary metabolites. *Glycyrrhiza uralensis* Fisch hairy root cultures were shown to produce more flavonoids when the over-expression of the Chalcone isomerase gene was

combined with either PEG8000 (2%) alone, yeast extract (YE) (0.1%) alone, or both of them as an elicitation treatment. Following this, the total flavonoids were extracted and quantified. According to Zhang et al. (2009), the findings demonstrated that during a three-week culture period, the total flavonoids accumulated by the wild-type hairy roots, the control transgenic hairy roots, and the double-treated transgenic hairy roots were 0.842, 1.394, and 2.838 (g/100 g DW), respectively. By over-expressing the salutaridinol 7-o-acetyltransferase gene via *A. rhizogenes*-mediated transformation, increased morphinan alkaloid production was seen in hairy root cultures of *Papaver bracteatum*. Through overexpression of Chalcone isomerase in hairy root cultures of *Scutellaria baicalensis*, the enhancement of flavone levels was investigated (Park et al. 2011). Acquiring an understanding of every route could facilitate the process of growing hairy root cultures in fermenters using different combinations of elicitors. Therefore, more secondary metabolites can be produced by applying elicitors to the hairy roots.

Hairy root culture mediated by *Agrobacterium rhizogenes*

The majority of agrobacterium species are pathogenic; they cause tumors and hairy roots in plants through the integration of pathogenic genes encoding enzymes that synthesize opine and phytohormones into T-DNA. *A. rhizogenes* strains exhibited varying degrees of hairy root induction efficiency. A4, 15834, LBA9402, MAFF03-01724, R-1601, R-1000, and TR105 are among the *A. rhizogenes* strains that are typically used to induce hairy roots in medicinal plants. The LBA9402 strain of *A. rhizogenes* was found to be the most effective in inducing hairy roots from *Linum flavum* leaf discs, although the other strains varied greatly in this regard. The host determines which *A. rhizogenes* strains are best for inducing hairy roots. For example, the A4 strain was found to be extremely virulent, but it was not successful in producing hairy roots on *Linum flavum* leaf discs, despite being highly effective in inducing hairy roots on many other plant species (Lin et al. 2003).

***Agrobacterium rhizogenes*' Ri plasmid**

Toxin-inducing (Ti) or retinoid (Ri) plasmids are present in virulent strains of *Agrobacterium*. The Ri plasmid (Fig. 2), which has distinct gene segments, is present in *A. rhizogenes*. When the transferred DNA (T-DNA) is found on the Ti or Ri plasmid, it is referred to as the T-region. A portion of the bacterium's DNA is transferred to the plant cell during *A. rhizogenes* infection. This DNA fragment is a duplicate of a section known as T-DNA. Encoding functions for Ti/Ri plasmid conjugation, opine synthesis, catabolism, and the initiation,

transfer, and integration of the T-DNA, T-DNA is a component of the approximately 200 kb Ti/Ri plasmid found in *Agrobacterium*. Approximately 10-30 kbp make up the T-regions on native Ti and Ri plasmids. T-DNA border sequences demarcate T-region boundaries. Largely homologous in sequence, these borders span 25 bp. In a straight repetition, they encircle the T-region (Gelvin 2003). Depending on the opiates they produce, ri plasmids can be categorized. While two regions (TL-DNA and TR-DNA) have been identified in the octopine (pTi) and agropine (pRi) plasmid types, a single T-DNA has been found in the nopaline Ti plasmid, mannopine, and cucumopine Ri types. There is roughly 15 kb of non-transferred DNA separating the two T-DNAs. The rootsucing (*rol*) genes are found in the central, less conserved section of the agropine T-DNA TL-DNA.

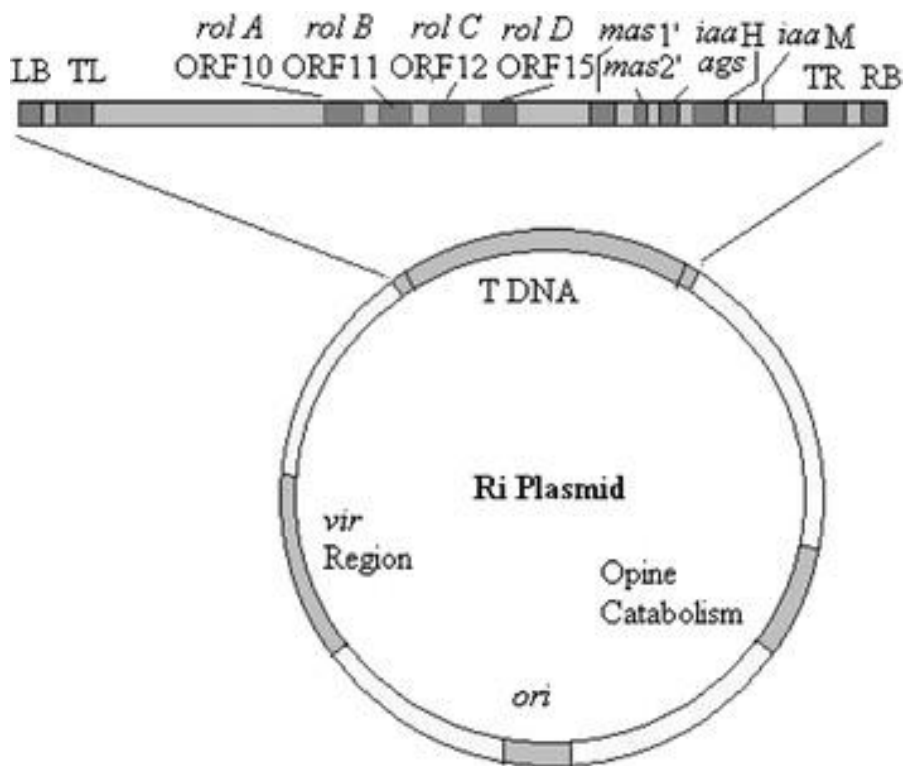


Fig. 2. Schematic representation of Ri plasmid of *A. rhizogenes*

The *rol* genes are found in the central, less conserved region of the agropine T-DNA TL-DNA. Agropine (*ags*) and mannopine (*mas10* and *mas20*) are two opiates that are synthesized from TR-DNA, and two genes, *iaaM* and *iaaH*, are responsible for the biosynthesis of auxins. The integration of TL-DNA and TR-DNA into the host plant genome occurs independently of each other, but TL-DNA transfer is necessary for the induction of hairy root syndrome, whereas TR-DNA transfer does not cause roots to form from transformed cultures.

A look at how the genes rolA, rolB, and rolC affect secondary metabolism

The rolA, rolB, and rolC oncogenes of *Agrobacterium rhizogenes* have long been recognized as modulators of plant growth and cell differentiation. The Solanaceae, Araliaceae, Rubiaceae, Vitaceae, and Rosaceae families of transformed cells may have their secondary metabolism activated by these rol genes. Research was conducted on the individual and collective effects of rol genes on secondary metabolism (Shkryl et al. 2008). The rolC gene alone has the ability to increase the synthesis of ginsenosides, pyridine alkaloids, indole alkaloids, and tropane alkaloids in transformed plant cell cultures. The genes rolB and rolC stimulate the synthesis of stilbenes and anthraquinones. It was also shown that the rolA gene stimulates the production of nicotine. But in transformed ginseng calli, rolA and rolB were unable to increase the production of ginsenosides. Similarly, in rolC transformed callus cultures of *Eritrichium sericeum* and *Lithospermum erythrorhizon*, there was a decrease in the production of caffeic acid metabolites. Research has also been conducted to determine the role that rol genes play in the synthesis of secondary metabolites (Bonhomme et al. 2000). *A. tumefaciens* carrying rolC and npt II genes transformed the first series of *Atropa belladonna* hairy root lines used in this study, while rolABC and npt II genes transformed the second series. The production of hyoscyamine and scopolanine was assessed following 3 and 4 weeks of culture in order to assess the potential involvement of the rolC gene in the formation of tropane alkaloids. The hairy root growth rate was significantly influenced (17-fold increase) by the rolC gene alone. But when the rolABC genes were combined, the growth rate of hairy roots increased significantly. Conversely, in *A. belladonna* hairy root cultures, the rolC gene by itself was just as effective as the rolABC genes combined (mean value of total alkaloids: 0.36% dry weight, i.e., 12-fold more than in untransformed roots) in promoting the biosynthesis of tropane alkaloids. There is a relationship between ginsenoside synthesis, tropane alkaloids, and *Catharanthus roseus* alkaloids and the expression of the rolC gene.

Significance of hairy root culture

The following are the reasons hairy root culture is important:

- Entire plants may be destroyed when roots are harvested in order to extract secondary metabolites. Consequently, there is now more interest in developing hairy root cultures in order to produce secondary metabolites.
- Hairy root cultures have the potential to grow more quickly without requiring an outside source of auxins.

- All hairy root cultures are stable in the production of metabolites because of their high genetic stability; in certain cases, they do not require light incubation.
- A variety of factors, including the carbon source and its concentration, the medium's ionic concentration, pH, light, temperature, and inoculums, can be optimized to change the yield in hairy root cultures.
- The production of secondary metabolites can also be enhanced by the application of methods such as precursor feeding, cell immobilization, elicitation, and biotransformation of hairy root culture.

Conclusion and future prospects

In order to overcome the restricted availability of physiologically active, economically valuable, and medicinally significant plant secondary metabolite compounds, metabolic engineering and biotechnological methods are being used as an alternative production system. Molecular mechanisms of T-DNA transfer, their interaction with host plant proteins, their role in plant defense signaling, and their integration into the plant genome for stable gene transfer for successful plant genetic transformation have all been successfully explored to date with remarkable success. Shape and plant host secondary metabolism are changed by T-DNA and the corresponding expression of rol genes. The technology for transforming plants has now advanced to a point where it can be sold. Secondary metabolism in plants is influenced by T-DNA and rol genes. Hairy root technology has advanced dramatically in the last few years in a number of areas, including the engineering of secondary metabolism, the increased accumulation and excretion of metabolites following elicitation, the generation of therapeutically recombinant proteins, the trapping of biomolecules released in the medium, and the scaling up of the culture procedure. It is simple for hairy roots to spread and change. These differentiated cultures offer significant advantages over cell suspensions due to their efficient productivity and genetic and biochemical stability. Private enterprises are starting to show interest in the hairy root system due to its enormous potential for phytoremediation and metabolite production. Hairy roots will soon give biotechnologists powerful tools to access the valuable subterranean resources found in the plant kingdom. This method is very helpful for preserving plants, particularly those that have roots as a vital organ that contain valuable medicinal metabolites. A primary factor in plant uprooting, root harvesting renders the plant rare. The biofermenter can produce the significant medicinal substances by employing the rhizogenesis technique in order for plant products to be easily accessible to human society and to be synthesized on a commercial basis. The information and methods discussed in this

chapter need to be helpful in refining methods for rhizogenesis and enhancing secondary metabolites in other significant plants.

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CHAPTER 9

Reverse genetics approach in crop improvement

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Abstract

Identifying, researching, producing, and utilising genetic variety are undoubtedly essential components of human growth. The origins of traditional, or forward, genetics can be found in the early attempts made by humans to domesticate plants and animals. Thousands of years after DNA was discovered to be the heritable material, reverse genetics—a technique that tests and uses functional variation in genes—was made possible by the recovery of specific lesions in the genome. The finding made at the beginning of the 20th century that mutations may be generated in genomes at frequency several orders of magnitude higher than normally seen in nature played a significant role in both forward and reverse genetics. Techniques like CRISPR/Casp, TALEN, RNAi are becoming popular day by day. Thousands of new crop cultivars have been developed as a result of the capacity to generate unique diversity. There are examples of enhanced yields, greater disease resistance, tolerance to abiotic conditions including salinity and drought, and better nutritional quality. Understanding the function of a gene and examining the effects of induced mutations or altered gene expression are the main objectives of reverse genetics. One useful technique that makes a clear connection between a gene product's role in vivo and its metabolic activity is reverse genetics. This chapter's goal is to go over the latest methods and applications of reverse genetics in plant breeding.

Keywords: Forward genetics, Reverse genetics, Mutation, TALEN

Introduction

When food production increases at a rate that is at least proportionate to the rate of population growth, we can refer to this as agricultural sustainability. 9.2 billion people will inhabit the earth by 2050, with the bulk of them residing in developing and least developed nations. This suggests that the countries with the fewest resources available for crop enhancement will face the greatest pressures on crop production. Plant breeding and agricultural biotechnology is the newest approach to solve different problems of agriculture to boost the production and productivity. Numerous biotic and abiotic stressors have a major impact on crop productivity

variations. The main abiotic stressors known to affect food production are acidity, salinity, and drought. Insects, illnesses, and weeds are examples of biotic variables that contribute to lower yields. Plant breeders are currently concentrating on creating enhanced cultivars that will give higher yields while also being able to withstand less-than-ideal soil and climate conditions. Many enhanced cultivars have reached the farming community through the use of varied breeding techniques, and these cultivars have played a crucial role in increasing global food production. Heritable biological variety is supported by genetic variation resulting from spontaneous natural mutation. Since Mendel's revolutionary work in genetics, studies have been carried out to determine the variation in DNA sequence creating the attributes that Mendel noticed. For instance, it was discovered that a transposon insertion in a gene encoding a starch branching enzyme is responsible for the round versus wrinkled phenotypic of peas (Bhattacharyya et al., 1990). It is believed that the difference between the phenotypes of tall and short pea plants results from an amino acid substitution caused by a single nucleotide mutation (Ellis et al., 2011). The traits in these two cases are fascinating, though not surprising, as they arise from two different types of mutations, insertions and point mutations, which would later be widely used in forward and reverse genetics.

A forward genetic screen, also known as forward genetics, is a method for determining which gene (or genes) in an organism is responsible for a given trait. The 1970s saw the development of recombinant DNA and sequencing technology, which made it possible to recover induced lesions that were responsible for the observed heritable phenotypic trait differences. As a result, everything needed for what is regarded as the standard forward genetic technique was at hand: (1) create mutations in a population; (2) recognise intriguing, recently-emerging phenotypes; and (3) take the necessary actions to clone the gene or genes responsible for the phenotypic variation. In order to understand how a gene functions, a technique known as reverse genetics looks at the phenotypic effects of certain altered gene sequences. Typically, reverse genetics works in the opposite manner from classical genetics' "forward genetic screens." Conventional genetics begins with a mutant phenotype, then clones and sequences the gene to determine its DNA and protein sequence, utilising progeny ratio analysis to prove the gene's presence. Though it works the other way, reverse genetics is a novel approach made feasible by recombinant DNA technology. In reverse genetics, a protein or segment of DNA lacking any genetic information serves as the basis for the creation of a mutant gene and corresponding mutant phenotype. Gene disruption, also referred to as gene knockout, and *in vitro* mutagenesis are crucial techniques for reverse genetics. Historically, the use of biological agents—more especially, transposable elements—

were the source of reverse genetics in crops. Transposons, which Barbara McClintock initially identified in maize, are able to freely migrate and insert into new areas of a genome. In this chapter we discussed different reverse genetics approach used for development of crops one by one by precisely.

Forward genetics vs reverse genetics

Gene function is ascertained by forward and reverse genetics methods. Forward genetics deals with identifying and characterising the gene that causes the mutant phenotype, whereas reverse genetics studies the effects of induced mutation or altered expression of a particular gene and attempts to understand the function of the gene. Forward genetics works from phenotype to sequence; reverse genetics works in the opposite way, when a gene's specific function is unknown despite its known sequence. Reverse genetics involves modifying or disrupting a particular gene or gene product, after which the phenotype is assessed (Fig 1)

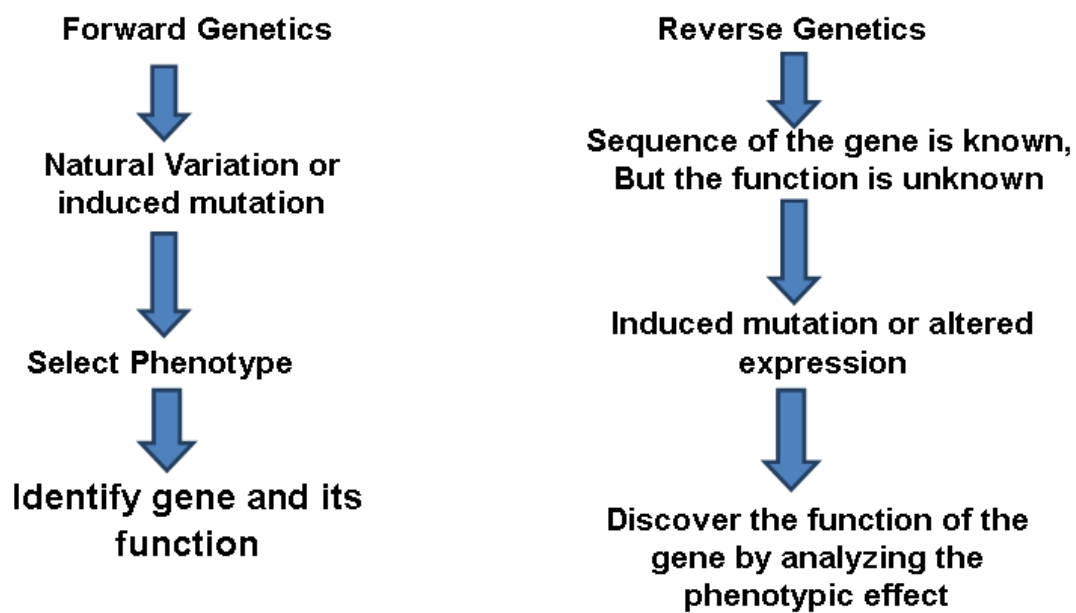


Fig 1. Difference between forward and reverse genetics

Reverse genetics have certain advantages over forward genetics. Compared to classical genetics, it takes less time. Furthermore, no prior understanding of gene function is necessary. In the case of reverse genetics, however, single allele mutations might be deceptive.

Different reverse genetics approaches

In reverse genetics, the aim is to determine the function of a gene and examine the effects of produced variation within it. The disruption or alteration process can be directed, as in the case of gene silencing or homologous recombination, or it can be non-targeted random

disruptions (e.g., chemical mutagenesis, transposon mediated mutagenesis) followed by screening a library of individuals for lesions at a specific location.

In contrast to non-targeted gene disruptions brought about by transposon-mediated and chemical mutagenesis, two popular methods for targeted gene mutation are homologous recombination and gene silencing. Researchers can obtain TDNA insertion mutants for model plants like *Arabidopsis*, as they have been generated (Krysan et al. 1999). Different reverse genetics approaches are discussed briefly here.

1) Large-scale random mutagenesis and screening

Employ forward mutagenesis (such as EMS), but check for nucleotide alterations in your target gene rather than a specific phenotype. This is accomplished by running PCR on the desired gene and observing minute variations in the PCR product's migration across a gel or column. Although it is theoretically possible to sequence each person's DNA and search for variations, more effective detection techniques exist now a days like DHPLC (Denaturing High Performance Liquid Chromatography), DGGE (Denaturing Gradient Gel Electrophoresis), SSCP (Single-Stranded Conformation Polymorphism).

2) Homologous recombination:

Recombination is the process by which DNA molecules exchange genetic information; homologous recombination occurs when the exchange occurs between homologous DNA molecules. This technique functions on yeast, bacteria, mice, and other mammals. It has been possible to eliminate all anticipated ORFs in yeast using this technique. A yeast deletion set, comprising around 6,000 yeast strains with individual genes deleted, is available for purchase. This technique has been used to knock out numerous mouse genes. *Drosophila* has a freshly established reverse-genetic mechanism based on homologous recombination. Although it takes a while to complete and calls for the creation of certain transgenic flies, it shows promise (Stemple, 2004).

It is now possible to target genes in rice by homologous recombination. With the addition of site-specific recombination systems (such as Cre-lox), the future of homologous recombination as a common method for reorganising the rice genome and maybe those of other plants looks promising. (Iida and Terada 2004).

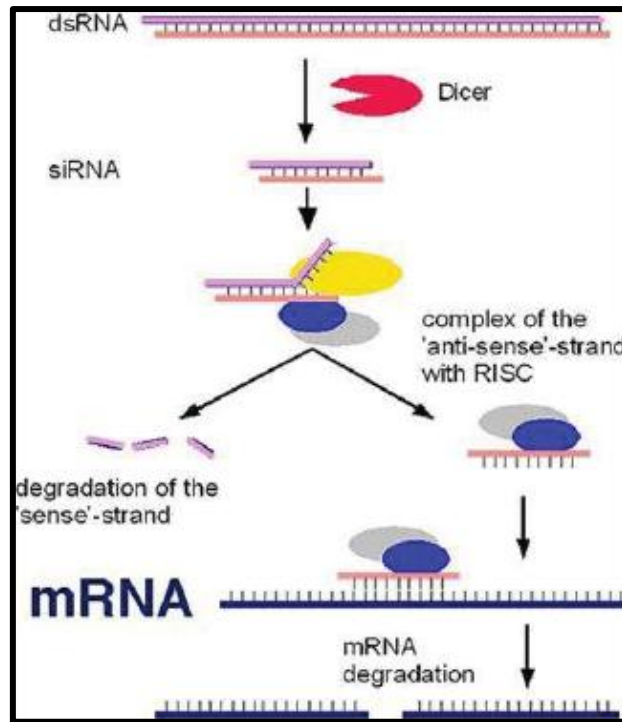


Fig 2. Mechanism of RNAi technology (Lewin and Lewin, 2004)

3) Gene silencing:

Antisense and sense RNAs can block the expression of a target gene through a process known as RNA interference (RNAi). It operates on the principle that complimentary sequences can be recognised and destroyed by double-stranded RNA (Lewin 2004). Double-stranded RNA was found to be an effective technique for suppressing gene expression, leading to the discovery of RNAi in *Caenorhabditis* worms (Fire et al. 1998; Kuttenukeuler and Boutros 2004). Reverse genetics has an intriguing new technique called RNAi-based silencing. Recently, RNA interference has developed into a potent technique for silencing gene expression and examining the loss-of-function phenotype. This makes it possible to analyse gene function in situations when mutant alleles are unavailable. It was once referred to as quelling, post-transcriptional gene silencing (PTGS), and co-suppression. Sometimes RNA interference (RNAi) only slightly suppresses target gene expression rather than totally eliminating it. Instead of using the term "knockout," "knock down" is used in these situations. Double stranded RNA can be cut by a dicer to create small inhibitory RNA (siRNA). To target and destroy mRNAs, a siRNA can be converted to a single strand anti-sense RNA. Several oval-colored proteins are needed for effective RNA interference. "RNA induced silencing complex (RISC)" was the name given to the protein-containing complex. A comparable RNAi mechanism may also be responsible for the phenomena of post-

transcriptional gene silencing seen in plants (Waterhouse et al. 1998). The process of gene silencing is given in Fig 2.

4. Genome editing:

Numerous techniques have been created to direct mutations to a particular region of the genome. Some popular gene editing techniques are discussed below:

a) CRISPR/Cas9 Genome Editing Technology

The ability to modify nearly any genomic sequence with a known Protospacer Adjacent Motif (PAM) to achieve a desired phenotype is made possible by modern advances in molecular genome editing technology, such as the Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR/Cas9), an RNA guided endonuclease, which has already solved major challenges in crop improvement, such as plant disease and insect pest resistance, tolerance to climate changes, yield and quality improvement, etc. in nearly 20 different plant species. A 20 bp long single guide RNA (a tracrRNA-crRNA chimaera) is used in the CRISPR/Cas9 system. It is designed to bind to the target gene in a complementary sequence. Cas9 is a nuclease that breaks DNA into single or double strands. These breaks are subsequently filled up by the cell's own DNA repair machinery through the use of Non-Homologous End Joining (NHEJ) or Homologous Directed Repair (HDR) mechanisms when donor DNA is present (Cui et al., 2018). Compared to the time-consuming back crossing method of conventional breeding, plant breeders have a remarkable potential to swiftly improve an elite genotype for numerous traits utilising the CRISPR/Cas9 system. This system is successfully used in yield improvement of rice, quality improvement in rice (biosynthesis of 2-acetyl-1-pyrroline) and wheat grain (low gluten content). Biotic and abiotic stress tolerance in rice and tomato. Effective transport of CRISPR/Cas9 machinery into precise plant cells, plant regeneration, and expression in viable plants present significant challenges, particularly in the agricultural sector. There is an urgent need for a novel delivery strategy, such as direct administration to plant apical meristems or pollen grains, in order to achieve high efficiency genome editing in plants.

b) Transcription activator-like effector nucleases (TALENs)

TALEN (Transcription Activator-like Effector nucleases) is a potentially useful genome editing tool that can target any DNA sequence in a wide range of organisms. The TALE (Transcription Activator-like Effector) protein, which alters the host plant's gene transcription to increase virulence, was found to be present in the plant pathogenic bacteria *Xanthomonas* spp. for the first time. A characteristic type III secretion system and bacterial colonisation in the host are required for TALE inoculation. When paired with nuclease, TALE may cleave

double-stranded DNA and bind to any DNA sequence. Following another disruption, homology-directed repair (HDR) or non-homologous end-joining (NHEJ) is used to repair DNA. By carefully altering the sequence, NHEJ causes an induced gene mutation.

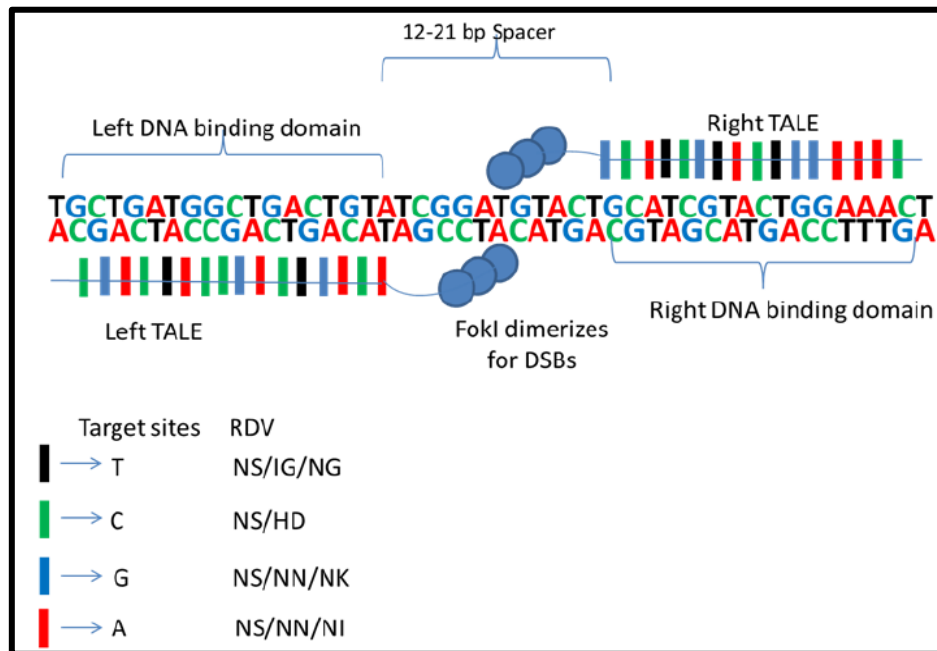


Fig 3. Schematic illustrations of TALEN construct and function

Plant genome modifications using TALENs have been widely applied for any form of enhancement. In the current era of genome editing, the base specificity of TALENs is very accurate, which aids biologists in minimising off-target genome modifications. Because of its degeneracy, when it comes to preventing mutational alterations in the genome, TALEN is thought to be the most dependable genome editing technique currently in use. Plant breeding has made considerable use of TALENs to increase yield, resilience to biotic and abiotic stresses, tolerance to herbicides, nutritional quality, and shelf life. Rice types resistant to *Xanthomonas oryzae* pv. *oryzae*, which causes bacterial leaf blight, were the first crop to be edited by TALEN. Sugarcane's biofuel quality has increased due to a TALEN-induced mutation in the gene encoding caffeic acid O-methyltransferase (COMT), all without affecting biomass or stress tolerance. These were the first TALEN-based genome-edited commercial varieties of soybean, with two fatty acid desaturase genes modified to boost oleic acid content and decrease harmful trans-fat (linoleic acid) content. In rice and brassica, TALENs-induced mitochondrial gene editing also overcame cytoplasmic male sterility (CMS). TALEN construct and its function is summarized in Fig. 3

c) Zinc Finger Proteins (ZFPs)

In the current biotechnological era, precise targeted genome editing (additions, deletions, or substitutions) technologies like Zinc Finger Proteins (ZFPs) have increased the possibility of improving crop resistance, adaptation, quality parameters, and yield. ZFPs have been used as zinc finger protein transcription factors (ZFP-TFs) or zinc finger nucleases (ZFNs) in plant biotechnology. The modular structure of zinc finger proteins (ZFPs) offers a seductive framework for the synthesis of ZFNs. These techniques often make use of site-directed nucleases (SDNs), which are composed of nuclease domains joined to DNA-binding domains (ZFPs). Based on the residues in the α helix, each designed zinc finger (ZF) with conjugated Cys2His2 motifs recognises a specific 3-bp DNA sequence. In order to identify a specific DNA sequence (12–18 bp), four to six different ZFs are frequently linked together. To cleave double-strand DNA, the FokI nuclease dimer cleaves specific DNA sequences consisting of 24–36 nucleotides, which requires the targeting of two ZF proteins. The DNA-binding domain ensures selectivity to specific DNA sequences, whilst the nuclease domain breaks double strands of DNA at the targeted location. The DSBs created by these reagents are repaired by the host repair pathways, which include homology-directed repair (HDR) and nonhomologous end joining (NHEJ). ZFNs have been routinely used to alter the genomes of many crops over the past 20 years (Petolino et al., 2015). Plant species such as Arabidopsis, Nicotiana, maize, petunia, soybean, rapeseed, rice, apple, and fig have all been genetically modified using ZFNs. Considerable advancements in improving the effectiveness of transgenic trait stacking have been made by means of a system that facilitates selection for ZFN-induced gene targeting by means of nuclease-mediated cassette exchange (NMCE). Fig 4 illustrates the anatomy of typical ZFP constructs.

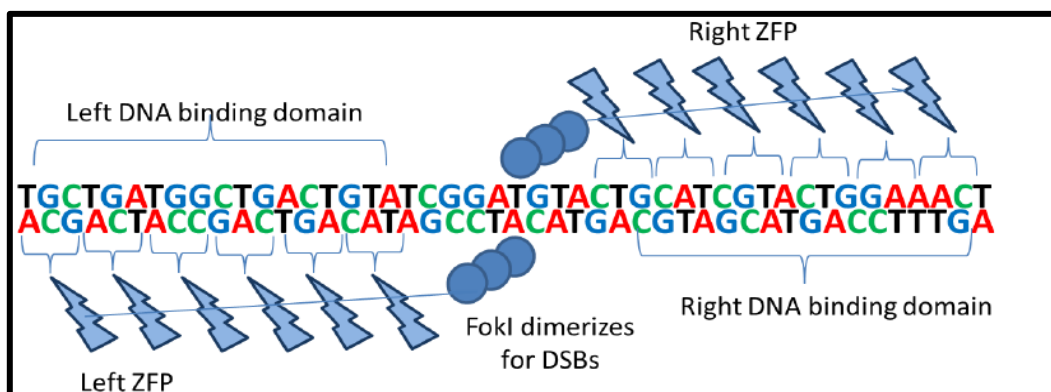


Fig 4. Structure of a ZFP construct

5. TILLING and ECO TILLING

Utilising mismatch-specific restriction enzymes, TILLING is a non-transgenic reverse genetics method that can identify naturally occurring or chemically induced mutations in target genes. The TILLING technique has undergone significant progress over the past 20 years, and the addition of Next Generation Sequencing (NGS) has ultimately elevated the process to a critical position in crop improvement. It is convenient to induce favourable point mutation using rapid high throughput TILLING, regardless of the crop's genome size, ploidy level, or mode of reproduction. Even in the CRISPR/Cas9 or TALENS age, TILLING is still promising since it enables low-cost plant population phenotyping prior to field deployment and aids in the simultaneous targeting of many genome-wide genes (Irshad et al., 2020).

Regardless of the crop's genome size or ploidy level, the application of EMS can induce the emergence of novel alleles, including missense and truncation variations, producing an M1 population. Gene-specific PCR is performed on a combination of M2 generation DNA samples that have been placed on microtiter plates. An endonuclease, like CELI, which belongs to the S1 nuclease family of single strand-specific nucleases, is incubated with amplification products. When there is a discrepancy between the strands of wild-type and mutant DNA, CELI responds by cleaving the 3' side of the mismatched DNA, leaving the homoduplexes intact. An automated gel sequencing apparatus is used to electrophoresis cleaved fragments. After that, the gel patterns are deciphered using widely available commercial image analysis tools. With PCR fragments of one kilobase in length, this fast evaluation technique locates the mutations within an accuracy range of ± 10 base pairs. EcoTILLING could also result in the natural induction of SNPs and InDels in the genome. Several crop plants, including rice, wheat, corn, barley, sorghum, oats, peas, tomatoes, sunflowers, Arabidopsis, maize, lotus, barley, potatoes, soybeans and Brassica oleracea, have also responded well to TILLING. B. melon, rapa, etc. TILLING is beneficial for allele mining in several plants as well.

One of the pioneer plants where TILLING was initially used was Arabidopsis. TILLING has been employed to develop mutants of CONSTANS (CO) gene which is involved in regulation of flowering time. TILLING has successfully targeted several wheat genes associated with agronomically desirable traits, flour quality or resistance to powdery mildew, spike formation, carotenoid metabolism, plant height, gluten content, etc. Variants of the waxy gene (Wx) and the puroindoline genes associated to grain hardness, pin a and pin b, were shown to be induced by TILLING. To identify distinct genes linked to flowering time, drought tolerance, starch synthesis, agronomically significant features, or grain quality, the rice genome was first mutagenized and subsequently exposed to TILLING. Genes such as

R1A (late blight resistance gene), DREB (dehydration responsive element binding protein; stress related gene), PITA (membrane receptor protein responsible for blast disease resistance), RPLD1 (phospholipase related to stress biology, signal transduction, and protein trafficking), TPS1 (trehalose-6-phosphate synthase; related to abiotic stress tolerance), and EXTE (related to plant growth) were mutated and subsequently studied for function analysis (Till et al., 2007).

Limitations of reverse genetics

Reverse genetics is not without its challenges, and not every organism can benefit from every technique. A number of factors need to be considered in order for it to be successful. Techniques that can be used without transformation, like TILLING, may be the sole viable option for species lacking effective transformation systems. The rate of mutagenesis is a crucial variable that can be challenging to quantify in certain situations. The genome cannot be so mutated that no mutant phenotype may be observed; rather, the load of mutations must be balanced with the recovery of mutants. The fertility of the mutated organism should also be taken into account, particularly in the first generation but also in generations that follow, both before and after mutagenesis.

Conclusion:

Reverse genetics, often known as a reverse genetic screen, examines an organism's phenotype after a known gene is disrupted. While reverse genetics aims to identify the phenotypes that occur from specific genetic sequences, forward genetics aims to identify the genetic foundation of a trait or phenotype. There's no doubting that newly discovered technology can significantly increase the efficacy of forward and reverse genetics for functional genomics and plant breeding. Using transgenic technology, reverse genetics modifies the function of a known gene. But because to breakthroughs in sequencing, reverse genetics is now used to identify the sequence that underpins a phenotype. The discovery that mutations can occur in genomes at frequencies many orders of magnitude higher than those typically observed in nature at the start of the 20th century played a crucial role in the development of both forward and reverse genetics. Thousands of new crop cultivars have been developed as a result of the capacity to generate unique diversity. There are instances of enhanced yields, greater disease resistance, tolerance to abiotic conditions including salinity and drought, and better nutritional quality.

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