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RESEARCH AND REVIEWS IN AGRICULTURE SCIENCE VOLUME III

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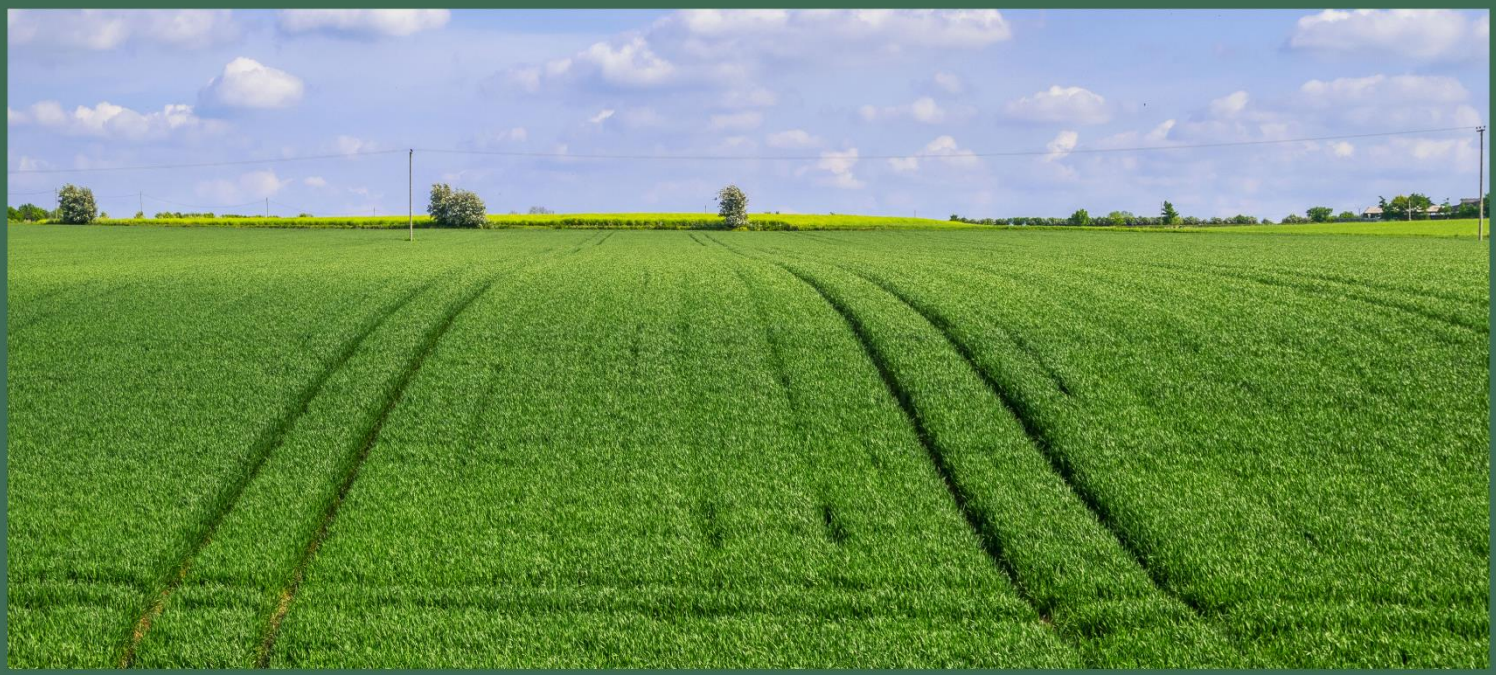

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PREFACE

Agriculture is the cornerstone of human civilization, providing sustenance, raw materials, and economic stability for societies around the world. As our global population continues to grow and environmental challenges become increasingly complex, the importance of agriculture in ensuring food security, environmental sustainability, and economic prosperity cannot be overstated. To meet these challenges, agricultural science has evolved rapidly, embracing interdisciplinary approaches, technology-driven solutions, and sustainable practices.

In this book, we have gathered a selection of research papers and reviews that delve into various facets of agriculture, from crop science and livestock management to soil health, agribusiness, and the integration of cutting-edge technologies. Each chapter represents a unique contribution to the ongoing dialogue in agricultural science, shedding light on emerging trends, innovative methodologies, and critical insights that have the potential to shape the future of agriculture.

The content within these pages spans a wide spectrum of topics, providing a holistic view of the challenges and opportunities that lie ahead in agriculture. Whether you are a seasoned researcher, a student embarking on a journey in agricultural science, or a stakeholder in the agriculture industry, we believe that this book will offer valuable perspectives and inspire further inquiry into the fascinating and ever-evolving field of agriculture.

We extend our gratitude to the authors who have generously shared their expertise and findings, as well as the reviewers who have dedicated their time and expertise to ensure the quality and rigor of the content presented here. This collaborative effort is a testament to the spirit of inquiry and the shared commitment to advancing agricultural knowledge.

As we navigate the complexities of feeding a growing global population, protecting our natural resources, and fostering sustainable agricultural practices, it is our hope that "Research and Reviews in Agriculture Science" will serve as a valuable resource and source of inspiration for all those engaged in the pursuit of a more resilient and sustainable future for agriculture.

Editors

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MODELING AND FORECASTING USING ARIMA AND LSTM

Gowthaman T*[1] and Sathees Kumar K[2]

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Introduction:

Time series forecasting is the process of projecting future values of a particular sequence using previous data. The most significant and rich sources of data for time series are the most current developments in sensor, computing, and communication technology. These developments alter how complex systems in the actual world are managed and observed (Chen *et al.,* 2015). For instance, stock price fluctuation series data are used by economists to forecast economic trends, area, production, and price time series data are used in agriculture to forecast, and atmospheric time series data are used by environmentalists to forecast environmental climate change. Time series forecasting, which aims to accurately predict future trends based on past and present time series data, is thereby becoming one of the key branches of big data analysis. (Shen *et al.,* 2019)

Time series develop chronologically and have high dimensionalities and temporal interdependence. Because of the high dimensionalities and temporal interdependence, even two numerically equal time points can belong to different classes or predict different behaviours. Time series can be classified as single variable time series or multivariable time series depending on the number of sample variables present at the time point. Correct time series forecasting is extremely challenging due to these complicated properties. In the past, linear statistical methods have been used to affect the time series forecasting problem in order to carry out forecasting tasks. Several practical nonlinear time series models, including the bilinear model, the threshold autoregressive model, and the autoregressive conditional heteroscedastic model, have recently been presented (Sathees *et al.,* 2023).

Since the last two decades, a number of Artificial Neural Network (ANN) algorithms have gained popularity and respect in the forecasting area after outperforming statistical methods in terms of prediction accuracy. Given the variety of ANN algorithms, choosing one for a given forecasting assignment should be based on a trade-off between three

factors: the difficulty of the solution, the level of prediction accuracy desired, and the characteristics of the data. When precision and complexity are taken into account, the Feed Forward NN predictor, which only allows input to pass through the network in the forward direction, produces the best results. However, on the addition of the third aspect, i.e. the data characteristics, Recurrent Neural Network (RNN) is found to be more suitable than FFNN. In RNN, the activations from each time step are stored in the internal state of the network in order to provide a temporal memory property. However, the most major weakness of RNN is carried out during the requirement of learning long-range time dependencies. To overcome this drawback, Hochreiter *et al.,* developed the Long Short-Term Memory (LSTM) algorithm as an extension to RNN.

Nassar *et al.,* (2020) used time series datasets of vegetables, fruits, and flowers to demonstrate the efficacy of deep learning models, LSTM, and CNN-LSTM in the accurate prediction of fresh product pricing for up to three weeks in advance. They accomplished this by contrasting the effectiveness of eight statistical and benchmark machine learning models with that of deep learning price prediction models. When Sabu and Kumar (2020) used time-series and machine learning models to estimate the monthly prices of areca nuts in the Indian state of Kerala, they demonstrated the effectiveness of the LSTM neural network. Gowthaman *et al.,* (2019) discovered that the deep learning method was the most effective one for forecasting the prices of agricultural products after contrasting the suitability of ARIMA and deep learning models on various datasets, including daily, weekly, and monthly data.

Data source

Considering the Delhi market, data series from the AGMARKNET website from January 2009 to December 2022 were gathered for the current analysis of the monthly wholesale price of bengal gram.

Autoregressive Integrated Moving Average (ARIMA)

ARIMA is a statistical analysis model that forecasts future trends using time series data. It maintains a form of regression analysis that attempts to predict future movements and random walks by studying the differences between values in the series rather than using actual data values. The lags in the differenced series are referred to as "auto-regressive," whereas the lags in the predicted data are referred to as "moving average." ARIMA (p, d, q) describes this model, where p indicates the order of auto-regression, d represents the degree of differencing, and q represents the order of moving average.

The inclusion of Autoregressive and Moving Average processes is more advantageous for achieving higher flexibility of actual time series data, which begins with the combination of autoregressive and moving average processes designated as ARMA (p,q).

ARMA (p,q) is indicated by

$$\emptyset(B)y_t = \theta(B)\varepsilon_t$$

where

$$\emptyset(B) = 1 - \emptyset_1 B - \emptyset_2 B^2 - \cdots \emptyset_p B^p$$

and

$$\theta(B) = 1 - \theta_1 B - \theta_2 B^2 - \cdots - \theta_q B^q$$

In which,

B - the backshift operator express by B(y_t) = y_{t-1}.

p – order of AR

q – order of MA

Box-Jenkins [9] Autoregressive Integrated Moving Average model developed by including "differencing" in the ARMA model which indicated by ARIMA (p,d,q) which is written as

$$\Delta^d Y_t = C + \phi_1 \Delta^d Y_{t-1} + \cdots + \phi_p \Delta^d Y_{t-p} + \varepsilon_t + \theta_1 \varepsilon_{t-1} + \cdots + \theta_q \varepsilon_{t-q}$$

In which, $\varepsilon_t \sim$ N (0, σ^2).

Recurrent Neural Networks (RNNs)

RNNs are a class of neural networks specifically designed for handling sequential data like time series. Unlike traditional feedforward neural networks, RNNs have a hidden state that allows them to maintain memory of previous inputs in the sequence. This memory mechanism enables RNNs to capture long-term dependencies and context information, making them well-suited for time series modeling.

The key components of an RNN are:

- **Input layer**: Receives the time series sequence as input.
- **Hidden layer**: Contains recurrent connections that allow the network to store and pass information across time steps. The hidden state at each time step is a function of the current input and the previous hidden state.
- **Output layer**: Produces the output predictions based on the hidden state.

The challenge of vanishing gradients in traditional RNNs, which hinders their ability to capture multiple time dependencies and long-term patterns, has led to the development

of gating mechanisms to replace activation functions. LSTM (Long Short-Term Memory) cells, for instance, incorporate three essential gates—the input gate, forget gate, and output gate—within each cell. These gates allow controlled adjustments to the cell's state, which are iteratively propagated to effectively capture long-term dependencies. This controlled information flow within the cell empowers the network to memorize multiple time dependencies, making LSTM particularly suitable for modeling long-term relationships.

Long Short-Term Memory networks (LSTM)

Hochreiter and Schmidhuber introduced LSTM, a specialised kind of recurrent neural network (RNN), in 1997. LSTM networks have been refined and improved over time in subsequent experiments, including those by Cho *et al.,* (2014). Speech recognition, language modelling, machine translation, image captioning, text recognition, and activity detection in video streams have all found successful applications for RNNs, including LSTM (Ullah *et al.,* 2017). LSTM networks excel at handling sequence dependencies among input data, making them ideal for sequence prediction tasks, particularly when dealing with complicated and nonlinear time-series data (Hsu, 2017).

Cho (2014) introduced the Gated Recurrent Unit (GRU) as an alternative to LSTM. GRU excels at predicting long-term dependencies while also effectively integrating short-term information. In contrast to the more sophisticated gating scheme and cell structure of LSTM, it has updated and reset gates. GRU permits the cell state to be changed and updated at each iteration via the reset gate, which provides more flexibility than LSTM's limited gradient change mechanism. Unlike GRU, LSTM keeps previous data and does not allow for complete data deletion. Chung *et al.,* (2014) discovered that cells having gating mechanisms, such as LSTM and GRU, outperform standard RNN cells that do not have gating features. Furthermore, extensive research conducted by Britz *et al.,* (2017) proved that LSTM outperforms GRU and other network configurations.

LSTM architecture: LSTMs address the vanishing gradient problem by introducing specialized units called "memory cells" and using a gating mechanism. The key components of an LSTM include:

1. **Cell state**: This is the long-term memory of the network. Information can be added or removed from the cell state through a series of gates.
2. **Hidden state**: The hidden state, also known as the short-term memory, is a function of the cell state and is emitted at each time step. It can be thought of as the output of the LSTM cell.

3. **Gates**: LSTMs have three gates: input gate, forget gate, and output gate.

   Input gate: Determines which values from the input should be added to the cell state.

   Forget gate: Controls which information from the cell state should be discarded.

   Output gate: Based on the cell state and input, it determines the hidden state that is emitted as the output.

4. **Training LSTMs**: LSTMs are trained using backpropagation through time (BPTT), similar to traditional RNNs. The gradients are calculated and used to update the network's weights, allowing it to learn from sequential data.

The LSTM gating mechanism filters out irrelevant input data and improves modelling accuracy for time-varying behaviours. The LSTM distinguishes itself by including a complex memory block in the hidden layer, which consists of four critical components: the forget gate, input gate, output gate, and memory cell (as shown in Figure 1). This architecture improves its capacity to effectively collect and model sequential dependencies. The mechanism of the LSTM can be simply concluded in the following phases at a given time 't'.

1. Decide the extent of information throw away from the output at last time step $h_{t-1}$and new input X_t at forget gate f_t:

$$f_t = \sigma(W_f[h_{t-1}, X_t] + b_f)$$

2. Determine how much information should be added to memory cell state C_t at the input gate i_t, and a candidate memory cell state C_t is updated:

$$i_t = \sigma(w_i[h_{t-1}, X_t] + b_i)$$

$$\widetilde{C_t} = \tanh(w_c[h_{t-1}, X_t] + b_c)$$

3. Update current memory cell state C_t using C_{t-1} and C_t:

$$C_t = f_t * C_{t-1} + i_t * \tilde{C}_t$$

4. Calculate the output h_t to next memory cell at output gate O_t:

$$O_t = \sigma(w_o[h_{t-1}, X_t] + b_o)$$

$$h_t = o_t * \tanh(c_t)$$

Where f, i, o, and C represent forget gate, input gate, output gate, and memory cell state, respectively. W, U, and b denote the input weight, recurrent weight, and bias of a certain hidden layer component, respectively. σ and tanh are the logistic sigmoid function and hyperbolic tangent function as activation function, respectively.

A neural network's output is determined by the activation function, also known as the transfer function, which is a mathematical formula responsible for forecasting neuron outputs. There are two types of activation functions: linear and nonlinear functions. Linear activation functions produce outputs that keep the input and output layers in a linear connection. A linear relationship, on the other hand, is frequently insufficient for actual applications involving complex information and different data kinds such as photos, videos, text, and sound.

Nonlinear activation functions, on the other hand, are critical for overcoming linear activation's limits. The Rectified Linear Unit (ReLU) and Leaky ReLU are two examples of nonlinear activation functions. These functions are classified as nonlinear because their slopes do not remain constant across all input values. ReLU, in particular, has a slope of zero for negative input values and a slope of one for positive input values.

Jarque-Bera test

The Jarque-Bera (JB) test, which was developed in 1987 by Carlos M. Jarque and Anil K. Bera, is used to determine how much a dataset deviates from normality. Skewness (S) and kurtosis (K), two important sample statistics, are used in this statistical test. The test statistic, abbreviated JB, is calculated as follows:

$$\text{JB} = \frac{n}{6}\left(S^2 + \frac{(K-3)^2}{4}\right)$$

Where, n- is the number of observations in the data sample. The Jarque-Bera statistic follows a chi-square distribution (JB $\sim$ χ^2) with two degrees of freedom for sufficiently large sample sizes.

McLeod and Li test

Based on the autocorrelations of the squared residuals, the McLeod and Li test (1983) is intended to test the null hypothesis of linearity against various forms of potential nonlinearity. The test statistic is given as:

$$Q = n(n+2)\sum_{i=1}^{h}\frac{r^2(i)}{n-i}$$

where $r^2(i)$ is the autocorrelations of the squared residuals and h is the number of autocorrelations.

Ljung Box test

The Ljung-Box test to assess the presence of autocorrelation within the residuals. The test hypotheses are defined as follows:

H_0: There is no autocorrelation in the residuals

H_a: There is autocorrelation in the residuals

$$Q^* = N(N+1)\sum_{i=1}^{k}(N-K)\rho_k^2\,(e)$$

Here, N represents the number of observations utilized in the model. This statistic Q* approximately follows a chi-square distribution with (k-q) degrees of freedom,

Model evaluation criteria

When a model performs equally well on training data from in-sample as it does on out-of-sample, it is said to be optimum. The following standards were used in this study to assess model performance:

a. Root Mean Square Error (RMSE)

It is the square root of mean square error and is also known as standard error values and written as:

$$\mathrm{RMSE} = \sqrt{\frac{1}{n}\sum_{t=1}^{n}\left(Y_t - \widehat{Y}_t\right)^2}$$

b. Mean Absolute Percentage Error

It is the percentage of average absolute error value and written as:

$$\mathrm{MAPE} = \frac{1}{n}\left(\sum_{t=1}^{n}\left\{\frac{Y_t - \widehat{Y}_t}{Y_t}\right\}\right) * 100$$

Results and Discussion:

The dataset of the bengal gram price series contains 168 observations, in which the first 156 observations have been used to for training and 12 observations have been used for testing the performance of the forecasting models.

Normality and Linearity test

When the data is non-linear and non-normal, the LSTM model can be used. The Jarque Bera and McLeod and Li tests were employed, respectively, to examine normality and linearity. The McLeod and li test confirmed the existence of nonlinearity in the price series, while the Jarque Bera test result showed that the series is deviating from normalcy. As a result, the LSTM model is suitable for predicting Bengal gram price series (Table 1). The ARIMA model is shown from its prediction performance point of view.

ARIMA model

Identifying the order of the models is the first and most important stage in the ARIMA. The straightforward method recommended in the literature is to determine

stationarity and compute the auto-correlation function (AFC) and partial auto-correlation function (PACF) for stationary series in order to find the best fitting ARIMA model. The results of an ADF test used to ascertain the stationarity of the price series are shown in Table 2. The price series for Bengal gram were found to be non-stationary. So, price series was converted into a stationary series using differencing.

The parameter 'd' was calculated by the number of differencing steps necessary to establish stationarity, and the parameters 'p' and 'q' were determined using the results of the PACF and ACF analyses of the stationary series. To determine the values of p and q, respectively, the partial autocorrelation function (PACF) and autocorrelation function (ACF) of stationary series were computed. ACF of the stationary series was cut off at the second spike and tailed off towards zero, whereas PACF of the stationary series also tailed off towards zero (Fig. 2). As a result, it was implied that the algebraic family of ARIMA on p=0, 1, 2; d=1; and q=0, 1,2; could have been utilized. Table 3 contains the results. By using the Ljung-Box (Q) test to determine if the residuals' independence was verified, the residuals' assumptions were verified. Among the all fitted models, ARIMA (1,1,2) gave less RMSE and MAPE in the testing set, though other models were given less RMSE and MAPE in the training set. Parameter estimate of ARIMA (1,1,2) is given in Table 4.

LSTM model

Unlike modeling using regressions, in time series datasets there is a sequence of dependence among the input variables. Recurrent Neural Networks are very powerful in handling the dependency among the input variables. LSTM is a type of Recurrent Neural Network (RNN) that can hold and learn from long sequence of observations. The algorithm developed is a multi-step univariate forecast algorithm.

Minimax normalization was applied to ensure data fell within the range of [-1, 1]. This preprocessing step maintained data consistency.

$$y_i = \frac{y_i^w - y_{min}^w}{y_{min}^w - y_{max}^w}$$

Where y_i is the monthly data; y_{min}^w and y_{max}^w the minimum and maximum values of the monthly data respectively. y_i are the normalized data. Single series datasets were transformed into lag value matrices, with a maximum of 12 lag values based on Auto Correlation Function (ACF) analysis. The 'MinMaxscaler' from the 'Sklearn' library used for data transformation and the LSTM models were implemented using Keras and TensorFlow library in a Python environment. Table 5 shows the values of optimum parameters of

LSTM. In LSTM a dense layer with one unit as output layer has been considered. Softmax activation function used with loss function as MAE and optimizer as Adam. The total number of epochs was considered as 225.

Comparison of ARIMA and LSTM

Forecasting ability of the both models was compared through the best ARIMA and LSTM model performance in the testing set. According to Table 6, the RMSE and MAPE values for bengal gram price series were lower in the LSTM model than ARIMA model, the features of pulses price series are difficult to comprehend because they exhibit volatile movement. The deep learning model LSTM is learned to capture the complex feature better than traditional ARIMA model. Forecast of next 12 months were obtained from the fitted LSTM model and given in Figure 3.

Table 1: Test for Normality and Linearity

Crop	**Jarque-Bera test**			**McLeod and Li test**		
	Statistics	P value	Inference	Statistics	P value	Inference
Bengal gram	94.54	<0.001	Non-Normal	51.26	<0.01	Non-Linear

Table 2: ADF test

Crop	**Actual Series**			**Difference Series**		
	Statistics	P value	Inference	Statistics	P value	Inference
Bengal gram	-3.10	0.11	Non-Stationary	-5.34	<0.001	Stationary

Table 3: Performance of different ARIMA model in training set and testing set and residual diagnostics

ARIMA	**Training set**		**Testing set**		**Ljung-Box test**	
	RMSE	**MAPE**	**RMSE**	**MAPE**	**Statistic**	**P value**
(1,1,1)	400.42	3.82	392.27	6.41	20.69	0.06
(1,1,2)	**399.77**	**3.86**	**391.24**	**6.40**	**19.74**	**0.07**
(2,1,1)	407.13	3.96	413.72	6.76	29.06	<0.01
(2,1,2)	403.00	3.85	364.82	5.90	23.15	0.03

Table 4: Fitted ARIMA model

Model	C	AR (1)	MA (1)	MA (2)	AIC	BIC
(1,1,2)	25.59* (9.76)	0.89** (0.03)	-0.41** (0.06)	-0.58** (0.06)	2322.12	2337.34

*Significant level at 5%, ** significant level at 1%

Table 5: Fitted LSTM model

No of layer	No of hidden unit	No of Epochs	Lag	Loss	RMSE	MAPE
1	25	225	8	0.0019	155.13	1.98

Table 6: Comparing ARIMA with LSTM

Model	RMSE	MAPE
ARIMA	240.26	3.25
LSTM	**155.13**	**1.98**

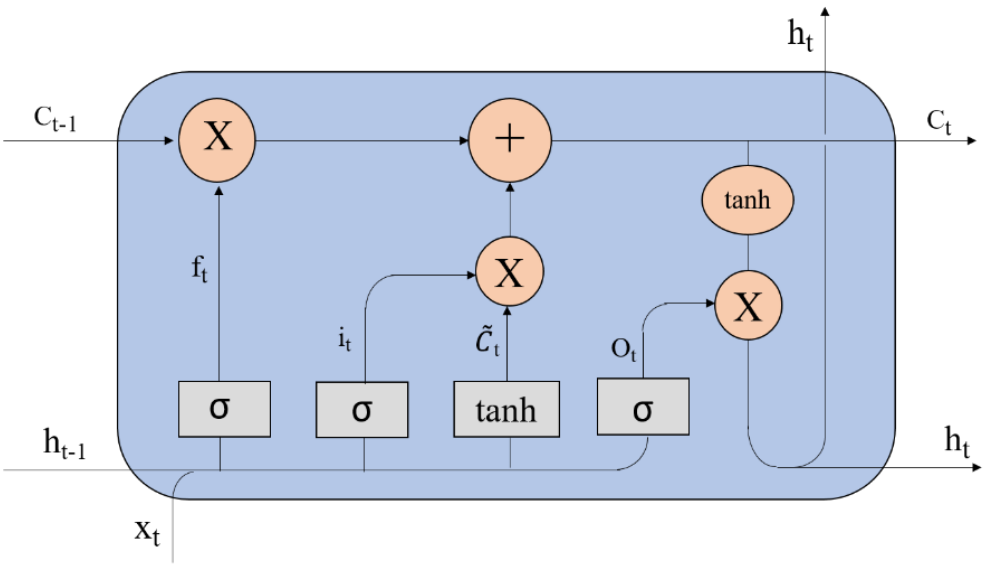

Figure 1: Structure of Long Short term memory

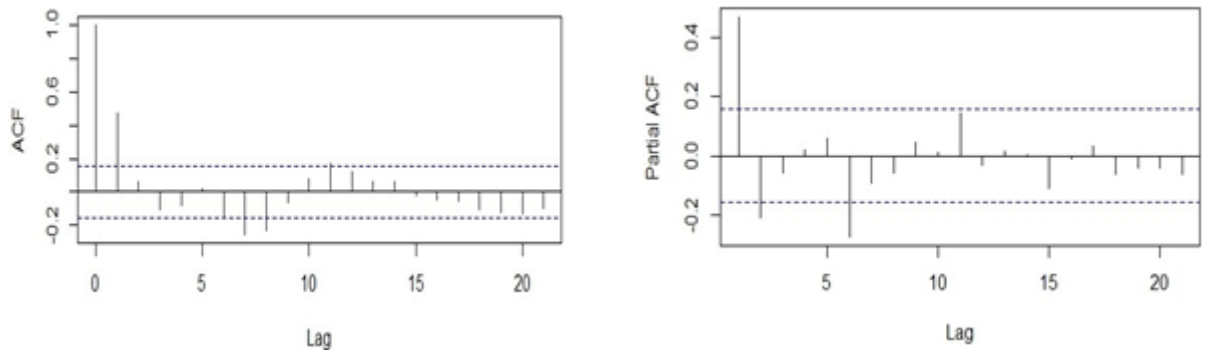

Fig 2: ACF and PACF of differenced price series

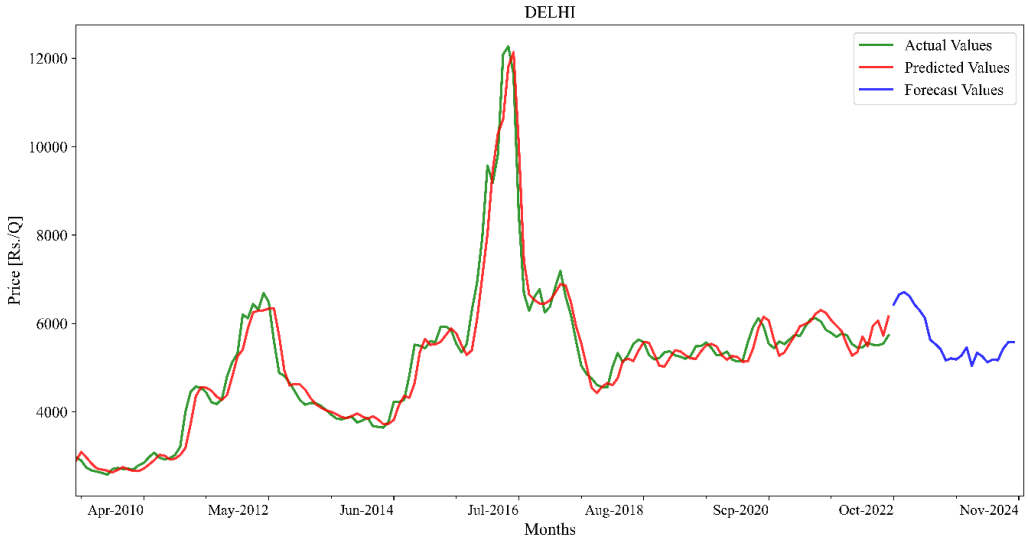

Figure 3: Fitted LSTM model along with forecasting values

Conclusion:

Based on the comparative study, we can conclude that the deep learning model LSTM captures the complex pattern of pulses price efficiently, obtains the highest prediction accuracy and outperforms ARIMA model. It has the potential to improve the forecasting accuracy significantly to the data that follows nonlinear and nonparametric. More research is needed to improve prediction accuracy by incorporating other important factors into the models.

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MICROGREEN FARMING

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Introduction:

Microgreens are veritably popular in recent times as a food supplement because of their high nutritive value and colorful characteristics. They're competitive shops with high nutrition and a short growth cycle. Primarily it's consumed in salads in utmost of the eatery also used in mists and sandwiches. It contains lots of minerals and has antioxidant properties. It can be grown with or without soil as well as using organic coprolites. The normal size ranges from 8 to 10 cm. Microgreens have the capability to cure numerous conditions because of their anti-cancer, antiseptic and anti seditious exertion. Some vitamins like K, C, E, lutein and beta carotene are present in microgreen and help to fight against cancer.

The term Microgreen was invented by Craig Hartman in the year of 1922.

Microgreens are edible vegetables which are older than sprouts and younger than babygreen. The level of nutrition differs according to the colour and growth stage of the plant. The level of phytonutrient in spinach is generally high. Lettuce microgreen had the loftiest antioxidant property and phenolic concentration as compared to the mature plant. The high concentration of Phenols are present in *Brassica* species.

Baby greens are 4-6 inches in height and require 21-40 days to grow but microgreens take a veritably short period to grow as compared to baby greens i.e 7-20 days and 4-6 inches in height. It can be grown in soil as well as in the hydroponic system. Filled with flavours and are used for garnishing. High amount of bioactive compound are present in microgreens as compared to fully grown plants [1].

Requirements for microgreen production

Microgreens seeds - The regular seeds cannot be used to grow microgreens. Only microgreens seeds can be used.

Pot/Tray - It is a vessel that can be used to grow microgreen plants should be 4 to 6 elevation deep.

Light source - Essential quantum of light needed by microgreens i.e at least 3 to 4 hours a day. Keep the pots in the area where the source of sunlight is good enough.

Water - It is one the important elements for microgreens. Soil should be kept wettish all the time.

Soil - Planting soil can be used to grow microgreen healthy and it should not contain any kind of pesticides, Chemicals etc. Mixtures of Garden soil, Coco peat, Cattle manure can also be used.

Method for growth

Choose the pots and trays that are at least 2-3 inches deep and as large in diameter as you want. Make holes at the bottom of the tray for drainage. For soil mix use seed starting soil or you can blend your own mixture by mixing 40% garden soil, 30% coco peat and 30% cattle manure or you can use leaf mold. Fill the pots with the soil mix and press it with the help of hands to make an even surface. The roots of microgreen do not reach that deep so 2-3 inches of soil should be good enough. Provide moisture to the soil by scattering water. After the soil is ready seed is spreaded on the surface of the soil evenly. Growing temperature is 6-32 degrees Celsius. Place the vessel in a spot where they get sunlight for 5-6 hours per day. Humidity is handled once a day using a spray bottle. Sprouts pop up in about 3-7 days. Once the seed is sprouted, it is necessary to provide moisture to the soil twice in a day using a spray bottle. After 4-6 days you will be thrilled to see your container full of the nutritious microgreen.

Note: keep the seed moist but not wet until the seed germinate. You can grow different seeds in the same container.

Microgreen is veritably precious to buy but it can be grown cost- effectively at home in a small area with simple supplements. The stylish time to gather microgreens is when the first set of true leaves are developed i.e. about 14- 20 days after planting. The position of nutrients in microgreen is 40- 50 times advanced than the other green. The flavour of the microgreen is much further lesser than mature vegetables because they're gathered at true splint stage.

Benefits of microgreen

- They contain lots of nutrients like vitamin k and fibre that help to maintain a high blood pressure also reduce the risk of heart diseases [4].
- Consumption of microgreens in daily life lower the risk of Alzheimer's disease [2].
- It has Anti-cancer and Anti-septic property [4].

- It also help to prevent osteoporosis and boost heart health [4].
- Daily consumption of microgreen help to reduce the inflammation in the body [2].
- Most of the microgreen containing high amount of beta-carotene that help in preventing diabetes and eye disease [2].
- It has the ability to reduce certain type of chronic disease such as Neurodegenerative disorder, Type-2 diabetes, Cardiovascular disease [2].

Types of microgreen

These are different types of microgreens are available –

- ❖ Red Amaranth- it is sweet in flavour and rich in vitamins such as A, C, K and minerals like ferrous, calcium, etc [8].
- ❖ Beetroot- it has reddish purple leaves which contain high amounts of vitamin A, B, and k.
- ❖ Cress- it is one of the traditional microgreen which can be grown having finely curled leaves. It is one of the good sources of vitamin C. It contains a high amount of sulphur.
- ❖ Broccoli- It is a highly nutritious microgreen that contains high amounts of protein and chlorophyll which help to stimulate our immune system. It is a power house of sulphur.
- ❖ Fenugreek- It is a very good source of Vitamin E and some minerals. It stimulates appetite and highly effective against anaemia and fatigue [10].
- ❖ Kale- This microgreen also known as the powerhouse of Vitamin C and it is antioxidant in nature also helps to prevent macular degeneration.
- ❖ Mustard- It help to stimulate blood circulation also highly effective against fever and cold and other type of poly nutrient like lutein and zeaxanthin [12].
- ❖ Linseed- It is rich in omega-3-fatty acid and has a spicy flavour. Omega-3-fatty helps to regulate brain function and heart function.

Vitamins presence in microgreen

- Vitamin C- It is also called Ascorbic acid which is an essential bioactive element for the functioning of the body. It has antioxidant properties that help in various metabolic processes in humans. In the year 2020 Di Bella *et al.,* said that the amount of ascorbic acid in microgreen is much higher in the growth stage than the other stage. Every stage the amount of ascorbic is changed. If the nutritional stress occurred in plant so that the amount of ascorbic acid is increases upto 87% [3].

- Vitamin E- It is one of the important fat soluble nutrients and acts as an antioxidant. It protects our body from the damage which is caused by free radicals. It also help to boost our immune system so that our body fight against invading bacteria and virus. It prevents blood clotting and risk of coronary heart disease. High amount of vitamin E is present in spinach, broccoli, corn and soybean. High consumption of vitamin E supplement help to prevent mental function [11].
- Vitamin K- It is one of the fat soluble vitamins that exist in two forms. One is phylloquinone which is mostly present in fruits, vegetables. It is one of the very essential components required for bone and vascular metabolism as well as in blood coagulation. The other is menaquinone that is mostly found in some animals' food. Examples of vitamin k are prothrombin and osteocalcin [11].
- Lutein - It has several beneficial effects on the human eye as it prevents macular disease that leads to blindness and vision impairment. It also decreases the risk of cancer and improves cardiovascular health. It is rich in antioxidant and anti-inflammatory properties. The structure of lutein is similar to other carotenoid having 40 carbon atoms and eight isoprene units. Presence of oxygen atom inside the structure making lutein polar carotenoids which is classified as xanthophyll [8].
- β-carotene – It can be converted into vitamin A in the liver to support. It is different from other carotenoids. It is an antioxidant in nature and acts as a radical scavenger. The absorption of beta carotene and vitamin A varies from person to person. It has an ability to protect against ultraviolet radiation and oxidative stress. It has anti-aging property [6].

Microgreen farming as the recent trend in agricultural sector

Due to food adulteration, people have to face a lot of problems like allergies, rashes on the body. Nowadays, different types of chemicals found in vegetables do a lot of harm to people. Due to these chemicals people are suffering from dangerous diseases. Farmers use pesticides, insecticide to protect vegetables, which is harmful to the human body. Chances of food poisoning by eating microgreens are very less and today's generation is shifting from sprouts to microgreens because there are very less chances of getting disease which is caused by sprouts [9].

Sprouts are grown in a humid and hot environment so the chances of bacterial growth are very high and this will lead to food poisoning. Microgreens have 40 times more nutrition than sprouts like broccoli and some microgreens have 10 times more nutrition.

Example: radishes. By eating microgreens, the body gets a lot of nutrients and the chances of food poisoning are very less because it is completely organic. Micro Green is alkaline in nature and it keeps the pH of the body alkaline. Due to which the chances of getting sick are greatly reduced.

There is a lot of demand for microgreens in today's era due to its high nutritional value. Because It take very short period to grow, people are not need to wait for long to harvest microgreens. Micro green does not require a large space to grow as it can be easily grown in a small area and a variety of plants can be grown even in a small area. The requirement of microgreen farming is less as compared to other farming practices. The material required is cheap and easily available in the market. There are many options, you can grow any variety of microgreens you like.

- Increased Demand: Microgreens are gaining popularity due to their health benefits and culinary appeal, so the demand is expected to continue rising.
- Sustainable Practices: Sustainable farming methods, such as hydroponics and vertical farming, will likely become more common to maximize space and resources.
- Specialty Varieties: Farmers may focus on growing unique and exotic microgreen varieties to cater to niche markets and restaurants.
- Automation: Adoption of automation and smart technology to optimize growing conditions and reduce labor costs.
- Local Production: Increased emphasis on local production to reduce transportation and ensure freshness.
- Organic and Non-GMO: Consumers' preference for organic and non-GMO microgreens may drive more growers to adopt these practices.
- Educational Programs: Training and education programs may emerge to help aspiring microgreen farmers acquire the necessary skills and knowledge.
- Health and Nutrition Research: Ongoing research on the nutritional benefits of microgreens may further boost their popularity.
- Culinary Integration: Microgreens will continue to be incorporated into diverse culinary dishes and beverages, expanding their presence in restaurants and home kitchens.
- Market Diversification: Microgreens may find their way into more packaged foods, snacks, and health products.

These trends reflect the growing interest in microgreens as a sustainable, nutritious, and versatile crop.

Different shades of microgreen represent a different features-

- Red colour of microgreens indicate that they contain high amounts of lycopene which is a powerful antioxidant.
- Yellow and Orange colours of micro greens indicate that they contain high amounts of carotenoids like beta carotene and lutein which convert into vitamin A by the body.
- Blue and purple colour of micro greens contain high amounts of anthocyanin which help to protect the cells from damage.
- Green colour indicates that they contain a wide variety of phytochemicals including carotenoids and saponins.
- Brown colour indicates that they contain high amounts of Allicin which has antibacterial and antiseptic properties.

Common pest and disease that can be affect microgreen farming

Common Pest

- Aphids: These small, soft-bodied insects feed on plant sap and can quickly multiply, causing damage by stunting growth and spreading diseases.
- Thrips: Thrips are tiny insects that feed on plant tissue, leaving silver streaks on leaves. They can also transmit plant diseases.
- Fungus Gnats: Fungus gnats are small flies whose larvae feed on plant roots, leading to poor growth and weakened plants.
- Spider Mites: These tiny arachnids can suck the sap from microgreens, causing yellowing and stippling of leaves.
- Whiteflies: Whiteflies are small, white insects that suck plant juices and can transmit plant diseases.

Common Diseases

- Damping-off: Damping-off is a fungal disease that often affects seedlings, causing them to wilt, collapse, and die. It's typically caused by overly moist conditions and poor ventilation.
- Powdery Mildew: Powdery mildew is a fungal infection that appears as white, powdery spots on leaves. It can reduce the plant's ability to photosynthesize.
- Root Rot: Root rot is a fungal disease that affects the plant's root system, causing root decay, poor nutrient uptake, and wilting of the microgreens.

Preventive Measures

- Sanitation: Maintain clean and sterile growing trays, equipment, and growing media to reduce the risk of disease transmission.
- Proper Ventilation: Ensure good airflow to prevent high humidity and reduce the risk of fungal diseases like damping-off and powdery mildew.
- Monitoring: Regularly inspect your microgreens for signs of pests or diseases, and take action promptly if you notice any issues.
- Biological Controls: Consider using beneficial insects like ladybugs or releasing predatory mites to control pest populations naturally.
- Organic Pest Control: Use neem oil, insecticidal soaps, or diatomaceous earth as organic pest control methods to manage common pests.
- Well-Draining Soil: Use well-draining soil or growing media to prevent waterlogged conditions that can lead to root rot.
- Crop Rotation: Rotate your crops to different areas to disrupt pest and disease cycles.

By implementing these practices and staying vigilant, you can help prevent and manage common pests and diseases in your microgreen farming efforts.

Pros of microgreens

Micro greens offer numerous advantages. They are nutrient dense containing higher concentration of vitamins, minerals and antioxidant compared to a mature plants. They also provide culinary versatility adding a burst of flavour and visual appeal to two various dishes. Additionally, micro greens are easy to grow in small spaces making them accessible for urban farming and home gardening projects.

Cons of microgreens

While microgreens have many benefits there are few considerations to keep in mind. First they have a short life which can be challenging for commercial distribution. Second, the cost of purchasing micro greens can be relatively high compared to other greens. Lastly there is a risk of contamination during the growing process requiring careful attention to hygiene and food safety processes.

Applications of microgreens

Micro green have a wide range of applications-

- ❖ They are commonly used as garnishes for salads, soups and main dishes adding visual appeal and flavour.

- ❖ They are so popular in juices and smoothies providing a concentrated dose of nutrients.
- ❖ Additionally micro greens can be used in sandwiches, wraps and even as a base for Sushi rolls offering a unique twist to traditional recipes.

Conclusion:

In conclusion, microgreens offer a range of benefits such as high nutritional value, culinary versatility and ease of cultivation. While there are some drawbacks to consider, the overall advantages make micro green a valuable addition to any diet or culinary repertoire. Whether you are a health conscious individual or a professional chef, exploring the world of micro green can elevate your culinary experiences and promote a healthier lifestyle.

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MINICHROMOSOMES IN AGRICULTURE

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Abstract:

To face the ever increasing demand of food crops there is an increasing need to develop new and innovative modern agricultural techniques that enhance the quantity of crop as well as the quality of the crop. Genetic engineering has changed the face of agriculture but some of the limitations such as gene stacking issue, complexity of insertion site, transgene position effect are observed, which can be overcome by use of minichromosomes in genetic engineering. Minichromosomes are small structures resembling chromatin with centromere, telomere, origin of replication and additional genetic material present in it

Introduction:

The rapidly growing global population presents a profound challenge to modern agriculture. To overcome the increasing demand of food crops there is an increasing need to develop new and innovative modern agricultural techniques that enhance the quantity of crop as well as the quality of the crop. Minichromosome or artificial chromosome technology is the next generation tool for genetic engineering of plants such as wheat, rice, corn, soybeans, cotton, and various fruits and vegetables (Birchler *et al.,* 2016). Minichromosome technology is used to introduce traits like disease resistance, improved yield, and enhanced nutritional content. Stable expression and maintenance of multiple transgenes in one genome can be achieved by this technology (Weichang *et al.,* 2007).

Preuss was the first to discover this technology in a laboratory in Chicago while working with Arabidopsis thaliana (Bhardwaj, 2021). Biofortification strategies can be expanded in a number of opportunities due to the potency of minichromosome technology. Double haploid breeding can be utilized in conjunction with other biofortification methods for rapidly transferring engineered minichromosomes as a unit to other lines which will significantly enhance the nutritional value of crops (Cody *et al.,* 2015).

Minichromosomes:

Minichromosomes are small structures resembling chromatin with centromere, telomere, origin of replication and additional genetic material present in it (Bhardwaj,

2021). These have been generated in both monocot and dicot plants. These are more easily recovered from B chromosomes in maize. Engineered minichromosomes have greatly contributed to crop biotechnology. With the help of mini-chromosomes transgenes can be maintained independently of endogenous chromosomes and then can be easily transferred among germplasm without the problem of linkage drag that is accompanied with genomic transgenes (Gaeta *et al.,* 2013).

Role in genetic engineering:

Genetic engineering has changed the face of agriculture but some of the limitations such as gene stacking issue, complexity of insertion site, transgene position effect are observed, which can be overcome by use of minichromosomes in genetic engineering. Minichromosomes allow easy and faster transfer of traits from one plant to another. Multiple sets of genes can be transferred onto a single engineered chromosome package. This allows gene stacking side by side on the same chromosome as well decreases the chances of segregation of novel traits (Bhardwaj, 2021).

Telomere mediated truncation:

One of the methods of generating minichromosomes is by transforming the telomere sequence in the cells. This was first carried out successfully in mammalian cells by producing small chromosomes consisting of centromeres, which were further modified by using homologous recombination. In plants it was demonstrated for the first time in maize where a selectable marker and other sequence required for minichromosome modification were inserted on the same construct (Birchler *et al.,* 2016).

Arabidopsis, barley and rice have also been documented for telomere mediated truncation. Mini-chromosomes can also be produced by mixing free telomere repeats with interested transgene by performing co-bombardment. It results in co-integration of the DNA sequences with right orientation where a transgene will be ligated to chromosomal break and the telomere on the other end will cleave the chromosome. This approach helps to overcome the hectic procedure of cloning repetitive telomere repeats into plasmid. It has been successfully demonstrated in rice and maize (Birchler *et al.,* 2016).

Combining minichromosomes with doubled haploid breeding:

Double haploid breeding is a technique that refers to producing haploid plants of target lines or new genotypes by different ways, further doubling the number of chromosomes of the generated plants in the developmental lineages which leads the flowers to self-pollinate. With only two generations of manipulation a completely homozygous progeny is the end-product. After observing that B chromosomes introduced

into maize haploid inducer lines can be recovered in haploid derivatives in the progeny, the idea of combining minichromosomes with double haploid breeding was inspired. Modern methods for development of new plant varieties rely heavily on DH breeding technology therefore combination of mini-chromosomes with DH breeding would complement the modern approaches. This combination would be able to transfer collection of transgene to other lines without long-winded introgession process and with no linkage drag (Birchler *et al.,* 2016).

This combined transfer system will serve as an evaluation tool for analysis of the reactions of different transgenes with different genetic history (Birchler *et al.,* 2016).

Production of minichromosomes:

De novo:

Assemble all parts of the chromosome using molecular cloning techniques The desired chromosomal content is then assembled in vitro. The desired contents of the minichromosome are transformed into the host. This approach is utilized in minichromosomes in maize (Bhardwaj, 2021).

Top-down:

This method exploits the mechanism of telomere-mediated chromosomal truncation. Truncation occurs by selective conversion of telomere sequences into the host genome. This insertion generates additional telomere sequences and initiates new synthesis (Bhardwaj, 2021).

For minichromosomes to function properly, they must contain the necessary components necessary for successful reproduction during cell division. All eukaryotic chromosomes must contain centromeres to form kinetochores, replication initiation sites to maintain correct chromosome numbers during cell division and telomeres to protect chromosome ends from separation. Interestingly, in plants, the telomere is the only component that can be synthesized, like the centromere. is epigenetic in nature and the origin of reproduction has not been identified. Therefore, minichromosomes must be created using endogenous centromeres and telomeres in the process known as the top-down method (Cody *et al.,* 2015).

In some cases, mini-chromosomes may be created by breakage of chromosomes by radiation. Also it can be generated from the B chromosome by a break-fusion-bridge (BFB) cycle initiated by a special translocation between the B chromosome and the short arm of the Chromosome 9. This translocated chromosome has a folded duplication of 9S; that has dicentric state in recombination during meiosis. Dicentric chromosomes form anaphase

bridges at meiosis II and break at random points between two centromeres. Broken edges fuse together and break later in the subsequent gametophyte generation. The B chromosome is created during the second mitotic division of pollen. Instead of separating the broken chromosomes, they are delivered over as one sperm and then to the next fertilized egg. So B9 Chromosomes become smaller and smaller over time lifespan of progeny plants. Minichromosome structure and its meiotic transmission rate over several generations were determined by B-specific centromere arrangement and knob-unit repetition. Cytological examination of somatic metaphase chromosomes in the root tip revealed the presence of dicentric chromosomes underwent BFB cycle and five stable dicentric minichromosomes were discovered in the collection of minichromosomes as the BFB cycle was stopped by inactivation of one of the two centromeres. A large number of monocentric minichromosomes were also recovered, probably due to failure of broken chromosomes growing together again (Weichang *et al.,* 2007).

However, these minichromosomes must be targeted with genes before they can be used in biotechnology, and homologous recombination is not yet efficient in plants. To overcome this obstacle, a telomere-mediated chromosomal truncation system was developed in maize to create engineered minichromosomes. It was a 2.6kb repeat of the Arabidopsis telomere sequence transformed into maize along with the causative marker gene **b**reaking of the chromosome at the site of integration. Depending on the direction of transgene integration, the shortened chromosome may or may not carry the transgene near telomere sequences. Large-scale genetic transformation resulted in minichromosomes containing transgenes from both truncated A and truncated B chromosomes (Weichang *et al.,* 2007).

Applications:

Mini-chromosomes have various applications in biotechnology, basic studies and agriculture. We can use these chromosomes as a self-reliant platform for foreign gene expression without any integration with normal chromosomes (Weichang *et al.,* 2007). Mini-chromosome systems could have more freedom of manipulation because the cassette for recombination can be easily recovered at many different sites in the genome. These mini-chromosomes can be used as a vector system for functional genomics. With the help of mini-chromosomes, resistant genes could be stacked on it so that to generate a crop that can be more resistant towards viruses, insects, bacteria, fungi and to develop plants with herbicide and pest resistance (Bhardwaj, 2021). These mini-chromosomes can be used as a vector system for functional genomics. Can be applied to produce stress tolerant plants.

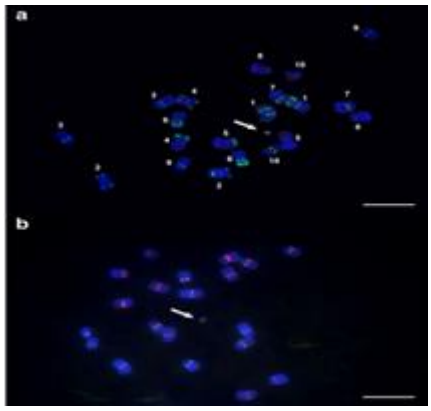
Mini-chromosomes can help to fulfill the global demands with the new modern generation of improved crops (Bhardwaj, 2021). Plants with engineered chromosomes or mini-chromosome can be used to produce multiple proteins and other metabolic products economically as compared to other methods (Weichang *et al.,* 2007).

Mini-chromosome in maize:

Maize minichromosomes are created by shortening both the normal A chromosome and the supernumerary B chromosome using telomere-mediated chromosome shortening. This approach uses a telomere-containing construct with a site-specific recombination cassette and a selectable marker to shorten the chromosome. During the integration process, the telomere-containing transgene closes the broken chromosome, triggering the generation of telomeres, leading to the generation of minichromosomes. The miniB chromosome is passed down reliably from generation to generation, but its abundance can be changed in the presence of a normal B chromosome. Foreign genes can be expressed through integration into the normal B chromosome and through the truncated miniB chromosome. Furthermore, the miniA chromosome does not pair with its progenitor chromosome during meiosis, suggesting a useful property of such constructs. This approach to constructing engineered chromosomes does not rely on species-specific cloned centromere sequences and can therefore be easily extended to other plant species. These platforms provide opportunities for the study of plant chromosome structure and function, as well as future developments in biotechnology and agriculture (Weichang *et al.,* 2007).

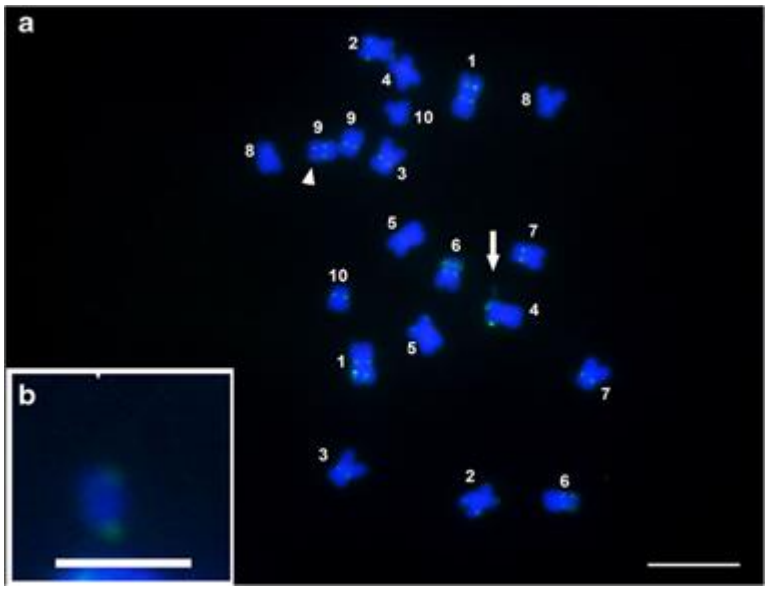
Modification of maize:

Modifications of genetically engineered maize minichromosomes were achieved in vivo using site-specific recombinases, specifically Cre recombinase, which removes the Bar selection gene and leaves a single loxP site. The engineered chromosome was obtained by telomere-mediated shortening and consisted of nothing more than a centromere and a transgene. Fiber fluorescence in situ hybridization technology was used to detect the transgene on the minichromosome inserted between the CentC centromeric repeat sections. This insertion was large enough to assume a tandem rise. This breakthrough will enable the development of customizable systems for crops that allow selective genes to be removed or new genes to be added. For eg. IGT-1 is a minichromosome which was modified to m-IGT-1 by Cre-recombinase mediated deletion of the Bar selection gene from IGT-1. Kryotype analysis of both were carried out with FISH probes to identify the modifications.



Karyotype analysis of IGT-1. a Karyotyping of IGT-1 using the FISH probes: TAG (green), CentC (green), NOR (green), and pGZ transgene (red). DAPI (blue) used for counterstain. Transgene signal and CentC were found in small centromere-sized chromosomes (arrow). None of the chromosomes were missing from the normal complement of chromosomes. **b** CentC (green) and CRM2 (red) were further used to probe IGT-1. These 2 probes co-localized to the centromere of the small chromosome. **Fig from**: (Gaeta et al.2013).

Karyotype analysis of mIGT-1. Karyotyping of mIGT-1 was done using the following FISH probes such as NOR (green), CentC (green), TAG (green) and pGZ transgene (red). The counterstain was used - DAPI (blue). Signals from transgene were rarely detectable on this chromosome. CentC found in small centromere-sized chromosomes (arrow). None of the chromosomes were missing from the normal complement of chromosomes. **b** Minichromosome were enlarged. Scale bars, 10 μm **Fig:** (Gaeta et al.2013).



Transmission of Minichromosome:

The mini-chromosomes produced by truncation of chromosomes A and B regained their normal centromere. This indicates that almost all mini-chromosomes are transmissible. Transmission rate of R2 can be comparable to that of early reported trisomic chromosomes. Meiotic transmission of type B minichromosomes varied between 12% to 39% through male parents and was comparable to transmission of miniB chromosomes generated by other means. Developments may introduce minichromosome pollen selection systems that may increase the transmission frequency by allowing only grains with minichromosomes to develop. Such a development will produce progeny in which all individuals will retain minichromosomes. Chromosome B and miniB chromosomes allow for dose manipulation. As an accumulation mechanism, maize B chromosomes are not separated during pollen mitosis and undergo preferential fertilization of eggs by B

chromosome-bearing sperm. To take advantage of this property, the long arm of the chromosome must be present in the same cell as the centromere and removed by truncation. Therefore, a truncated miniB chromosome without the distal long arm region exhibits normal segregation, but the non-segregating property can be restored by adding a full-length B chromosome to the genotype (Weichang *et al.,* 2007).

Table 1. Transmission of minichromosomes

Event	76-15a	86-4	86B23	86B155	R2
Chromosome size	1/2 B	1/5 B	1/16 B	3/4 B	NM
Transgene location	Distal	Distal	Distal	Distal	Distal
Cross	As male	As male	As male	Self	Self
Transmission (a)	14 of 36	3 of 25	7 of 29	9 of 18	15 of 46
Transmission (b)	30 of 36	6 of 25	8 of 29	16 of 18	16 of 46

Table from: https://doi.org/10.1073/pnas.0700932104

Stabilizing transmission of minichromosomes:

Minichromosomes do not behave like regular chromosomes. All small chromosomes in maize do not exhibit normal sister chromatid cohesion typical of meiosis I (MI). Therefore, sisters typically move from each other to the MI pole rather than together, resulting in random migration to the pole in the second meiosis. All studies on small chromosomes in maize suggest that if two copies are present, they are unlikely to find a mate in the early stages of MI. Therefore, the members of the pair classify themselves independently of each other, rather than separate from each other as usual. Therefore, several measures are needed to increase the frequency of minichromosome transmission. One solution to this problem in species with B chromosomes is to use a shortened B chromosome that is cleaved near the end of the chromosome so that its length causes it to behave like a normal chromosome. Another possible solution could be to place nuclear repair genes for gametophyte cytoplasmic male sterility (cms) on the minichromosome. In the cms background, the restorer allows only pollen grains with minichromosomes to survive within the male, so full transmission to the offspring is expected when mated with the female parent. A single minichromosome carrying such a restorer with a corresponding cms will reliably pass from one generation to the next. The cms-S cytoplasm of maize and the boro II cytoplasm of rice qualify to be used in this manner (Birchler *et al.,* 2016).

Recombination of Minichromosome:

Using an engineered minichromosome platform, a site-specific Cre/lox recombination system was developed on the R2 minichromosome, allowing site-specific recombination to occur within somatic cells. The site-specific recombination system was

shown to be a transgenic plant (J11-9) with a transgenic 35S lox66-Cre expression cassette at the end of the chromosome arm promoter without the lox71-DsRed gene. Provides Cre recombinase and an alternative lox recombination site to introduce and activate the 35S promoter. Recombination target sites lox66 and lox71 are mutant lox sequences that most favorably recombine within the transgene and produce red fluorescent protein by placing the promoter less than DsRed. The gene is under the control of the 35S promoter and adds genetic material to J11-9's minichromosome. Cre recombinase gene expression is also inactivated by recombination events. Events were screened by examining the red fluorescence of the primary roots of germinated seedlings and using PCR amplification with primers flanking the predicted recombination region. A total of 10 plants showed 120 red fluorescence. To confirm site-specific recombination, genomic DNA isolated from eight J11-9/R2 plants that expressed red fluorescence and was used as a template for PCR amplification across the amplicon was sequenced and lox recombination events, these were not passed on to the next generation due to self-pollination of the hybrid individuals, but among the 15 F2 siblings examined, only the J11-9 chromosome was present, or only the J11-9 chromosome was inherited. It was not possible to amplify the recombinant fragment in this individual. The red fluorescence of his chromosome 9 in the J11-9/R2 hybrid indicates that these terminal Lox sites are amenable to genetic manipulation by site-specific recombination systems. Lox because either the expression of his Cre recombinase in the sample was not high enough or the recombination site carrying bacteria could not be targeted by various genetic techniques previously demonstrated using this technique. Either the binding of the sites did not occur (Weichang *et al.,* 2007).

Minichromosomes for genome editing:

Minichromosomes could also be used as tools for genome editing. When CRISPR-Cas9 and the necessary guide RNA are introduced into the minichromosome and combined with DH breeding, the editing becomes as follows. Capable of replicating multiple haploid genes to capture the full spectrum Genomes with no linkage problems with or from edited transgenes The CRISPR-Cas9 machinery resides on small genetically engineered minichromosomes. Such chromosome pairs cannot undergo homologous pairing after doubling. As explained above, in meiosis it becomes part of the progeny The minichromosomes being edited are missing because they cannot be separated from each other. Therefore, the homozygous non-transgenic genotype is a normal set of genotypes with multiple edits remaining intact, but the edited minichromosome will be removed (Birchler *et al.,* 2016).

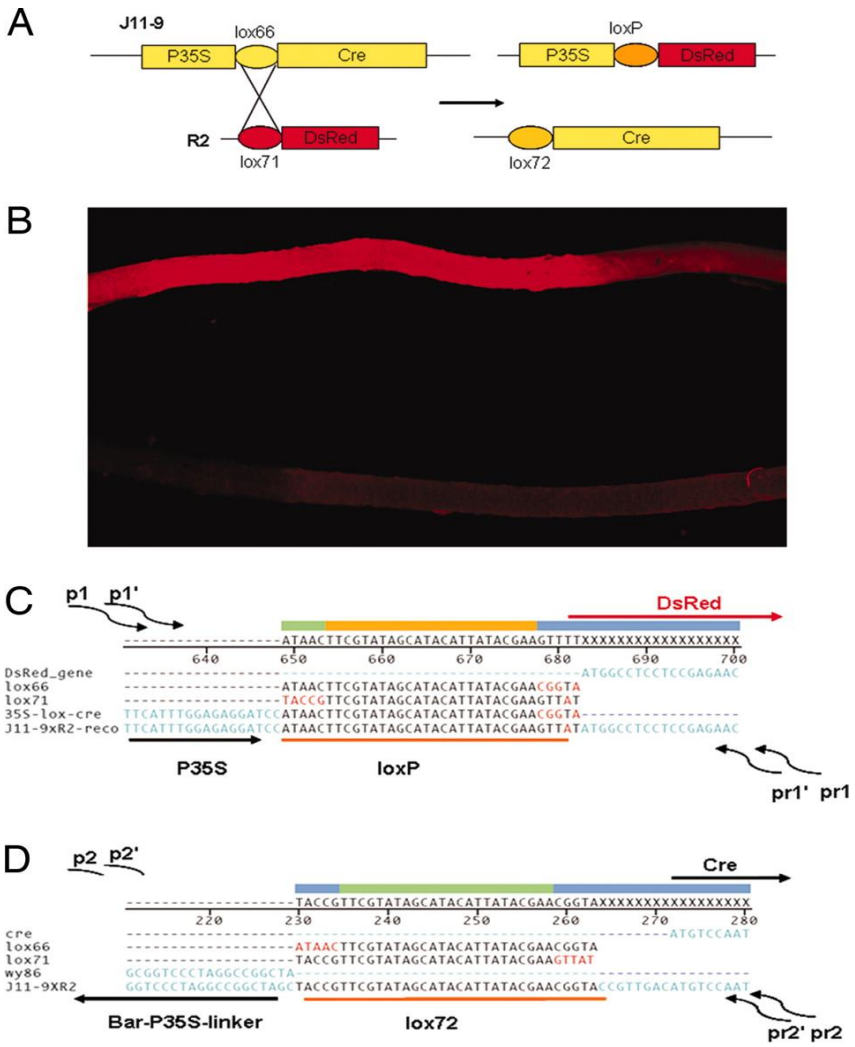


Fig from: https://doi.org/10.1073/pnas.0700932104 **Cre/lox-mediated site-specific recombination.** (*A*) Diagram of recombination between the R2 minichromosome (*lox71*-DsRed) and J11–9 (P35S *lox66*-Cre transgene). The CaMV 35S promoter (P35S), DsRed gene, Cre gene and *lox* sites (*lox66*, *lox71*, *lox72*, *loxP*) are shown. The recombination makes P35S-*loxP*-DsRed on the donor chromosome which activates the red fluorescence protein expression, activates *lox72*-Cre alongwith other sequences from the donor chromosome to the minichromosome. (*B*) Expression of red fluorescence protein from the recombination of J11–9 and R2 (root tissue) (upper) and no red fluorescence protein in the R2 control (lower). (*C* and *D*) Aligned sequence of the 2 recombination products amplified by PCR with sequences of DsRed coding region, *lox66*, *lox71*, and P35S-*lox66*- Cre (*C*) and the sequences of Cre coding region, *lox66*, *lox71*, and pWY86 (*D*). Outer primers for primary PCR - p1, p2, and pr1, pr2. Inner primers for nested PCR - p1, p2, and pr1, pr2. The DsRed gene (red arrow), CaMV 35S promoter (P35S, black arrow), Cre gene (black arrow), the linker region between P35S-Bar gene (black arrow), and the *lox72* and *loxP*, from recombination (red lines), are labeled.

Future scope:

Engineered minichromosomes can be used in all fields of future genetic engineering by either site-specific recombination with transgenes or genetic recombination of Minichromosomes containing additional foreign genes. It may also be used as transformation technologies for large DNA fragments used to facilitate the process to achieve large volumes target foreign genes. For example, both BIBAC and TAC conversion systems may allow this transformation of foreign DNA of more than 100 kb. Engineered minichromosomes include the potential for efficient gene stacking, which is considered a challenge for future plant biotechnology. Additionally, engineered minichromosomes can facilitate an understanding of fundamental questions about chromosomal structure and function, such as for centromeres, neocentromeres, B chromosome nondisjunction, as well as chromosomal behavior in general. By repetitive truncations, one might find the optimal minichromosome for genetic engineering (Weichang *et al.,* 2007).

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PLANT PARASITIC NEMATODES AND THEIR MANAGEMENT IN BANANA (*MUSA SP.*): A REVIEW

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Abstract:

Plant nematodes are abundantly found worldwide on Banana plants, which adversely affects crop production. There is a need to write a review article on the signs and symptoms of plant nematode because this help in the control and management. Assessing plant-parasitic nematodes involved in plant growth problems is significant. If so, it is necessary to control the nematodes to reduce or eliminate the damage they cause. One of the main reasons for low crop yields in the tropics and subtropics is infection by these microscopic roundworms. Nematode damage to crops is typically difficult to detect because many other variables restrict plant growth. This chapter aims to provide some light on plant-parasitic nematodes, the harm they cause, and how to control them.

Keywords: Plant Nematodes, Management and Banana.

Introduction:

Status of plant nematode on banana:

Banana is one of the non-commercial plants for cultivation one in backyard plantings and also commercial cultivation by private growers, all over the country local consumption for prayer by some people, and traditional pooja precrime as a temple. In addition, fruits have higher potential medical and pharmaceutical per potassium because of the cultivation of a plant slant. In India, an estimated 34.9 million metric tons of bananas were produced for the fiscal year 2023. This was an increase as compared to the previous fiscal year. The use of numerous enhanced technologies, such as the utilization of tissue culture plants, drip irrigation, and fertigation, has led to a rise in banana production. Even though we are the world's top producer of bananas, we will need to increase our output in the future to keep up with demand from a population that will only continue expanding. The production of bananas is hampered by numerous insect pests, nematodes, and diseases. Nematodes are one of the major production constraints.

Plant-parasitic nematodes are tiny worms that live mainly in soil and roots. In the case of banana plants, the most harmful species spend the majority of their lives in root and corm tissues. Plant parasitic nematodes, as pests of various horticultural and field crops, cause an annual monetary loss of Rs. 300 crores in Tamil Nadu alone. Namely, 50 species of plant parasitic nematodes have been reported in association with banana root systems (Parvatha Reddy, 1993) and are mainly responsible for controlling banana production to a greater extent (Koshy, Sundararaju and Sosamma, 1978; Rajendran, Naganathan and Vadivelu, 1979; Rajendra, Cannayane and Shobana, 2002 and Sundararaju 2005). Infestation by Plant Parasitic Nematodes is also a serious worldwide concern for banana cultivation.

Signs and symptoms of nematodes on banana plant:

Until they are well established, the majority of soil-borne plant pests and diseases are not visible above ground. Impaired water and nutrient uptake are the primary sources of the early symptoms induced by root-feeding bugs. Stunted plant growth, diminished vigour and production, early leaf loss, and a greater propensity to wilt or die back during dry spells are some of these signs.

Heavy infection results in stunted plants with narrow pseudostems and yellowish or discoloured bands running the length of the leaf blades (Milne and Kuhne 1968). Additionally susceptible to withering on moderately hot days are infected plants. Rabie (1991) claimed that the symptoms of "False Panama Disease," which resembles "Panama Disease," are brought on by the simultaneous presence of root-knot nematodes and different stress conditions, such as drought and freezing temperatures. The gradual withering back of older leaves, beginning at the tips, is one of the leaf symptoms. Galls develop on the primary and secondary roots, and after severe nematode infections, the roots can twist and occasionally split. In light of the fact that root-knot nematodes are nearly universally found in banana plantations (Daneel *et al.,* 2015), it is crucial to keep an eye on their population levels in immature crops. According to popular belief, these nematode pests can seriously harm young plants, resulting in suboptimal growth and production.

Major nematode pests of Banana:

Globally, more than 151 nematode species of 51 genera are known to be associated with Musa sp. (Gowen and Quenehenve 1990; Koshy and Gulsar Banu, 2000) causing an estimated 19.7% yield loss which is approximately amounts to US $ 17.8 million (Sasser and Freckman. 1987). In India, 71 species of Plant-parasitic nematodes are

known to be associated with bananas (Krishnappa and Reddy, 1995; Koshy and Sosamma. 2001).

1. The burrowing nematode, *Radopholus similis* (Cobb, 1893):

Cobb (1893) was the first to report the burrowing nematode seen in banana necrotic root lesions. The burrowing nematode was initially discovered in India in Kerala in 1966. The root rot and toppling diseases of bananas are caused by this nematode. Nematodes can reduce production by up to 41%.

Life cycle:

1. Females lay 4-5 eggs within infested tissues for two weeks**.**
2. Within 8-10 days eggs are hatched and then juvenile stages are completed in 10-13 days.
3. Females and all juvenile stages are causing infection.
4. Males are non-parasitic and without stylet.
5. Penetration occurs mostly near the root tip. The nematode penetrates within a day and cells around the site of penetration become brown. The stelar portion of the root nematode does not enter.
6. The nematode's life cycle is completed within 24-30 days at a 21-32 °C temperature.

Signs and symptoms:

1. Many plant species experience a progressive decline due to *Radopholus similis*, however, the symptoms are unique to bananas and plantain (*Musa* sp.). Plant uprooting (toppling) is the disease's most pronounced symptom in banana farms (De Villiers *et al.,* 2007).
2. Anchor roots are destroyed by burrowing nematode feeding, which also renders plants more prone to collapsing, particularly when fruiting or during severe gusts. Additional visible signs of root damage in bananas and plantains include slow sucker development, delayed fruiting, smaller fruit and lower bunch weight, as well as shorter plant life.
3. At the place of penetration and across the cortex, *Radopholus similis* destroys feeder roots and leaves reddish-brown lesions on larger root surfaces. Nematodes do not attack the central cylinder (stele), although they can girdle and kill roots in severe infestations. Blackhead disease gets its name from the eventual migration of burrowing nematodes from roots into the rhizome, which results in black, round lesions (Jones and Milne, 1982).

2. The root-lesion nematode, *Pratylenchus coffeae* (Zimmerman, 1898) T. Goodey, 1951:

Bananas are infected by various lesion nematode species, although Pratylenchus coffeae is the most common. All tropical nations that grow bananas have it.

Life Cycle:

1. Infested roots and corms provide adequate habitat for adult nematodes to survive.
2. The nematodes become active and lay eggs in the roots when the proper temperature and moisture are present.
3. Juveniles in their second stage hatch from eggs. J2 and others (J3, J4, and adult) invade the roots and move between them by passing through cortical cells.
4. Depending on the host, nematode species, and temperature, the nematode completes its life cycle in 28 to 65 days.
5. The development of the population is made easier by adequate soil moisture, low temperatures of 21 to 25 °C, and the availability of new, fragile roots *(Jonathan*, *et al.,* 2001 *and* Nickle, 1984).

Signs and symptoms:

1. Damage and signs by *Pratylenchus coffeae* and *R. similis* are extremely similar.
2. They include plant stunting, a slower vegetative phase, fewer leaves, a lower bunch mass, and a shorter lifespan for plantations. Although the producers continued to use sound management techniques, this occurred.

3. The root-knot nematode, *Meloidogyne incognita* (Kofoid and White, 1919) Chitwood, 1949:

The root-knot nematode species *Meloidogyne incognita* (Kofoid and White, 1919) Chitwood, 1949, and *Meloidogyne javanica* (Treub, 1885) Chitwood, 1949, are the two that are frequently associated with local banana plantations. On a banana in Zimbabwe, *Meloidogyne arenaria* (Neal, 1889) Chitwood, 1949, was discovered (Jones and Milne 1982). Root-knot nematodes were found in 93% of samples collected from commercial banana farms, according to a survey. The most prevalent nematodes were root-knot and spiral nematodes, which together made up 72% of the entire complex of plant-parasitic nematodes (De Jager *et al.,* 1999).

Life cycle:

1. Females lay about 200 to 300 eggs, which are deposited as egg mass in a gelatinous matrix just below the epidermis in the root cortex.
2. The juvenile in its following phase burrows into the extending root tip and creates a feeding location near the vascular region.

3. After 10 days of penetration, characteristic multinucleate giant cells begin to form. There are about 10 to 15 females in a single little root gall.
4. The life cycle is completed in 25-30 days *(Jonathan*, *et al.,* 2001 *and* Nickle, 1984)

Signs and symptoms:

1. Heavy infection results in stunted plants with narrow pseudostems and yellowish or discoloured bands running the length of the leaf blades (Milne and Kuhne 1968).
2. Additionally susceptible to withering on moderately hot days are infected plants. Rabie (1991) claimed that the symptoms of "False Panama Disease," which resembles "Panama Disease," are brought on by the simultaneous presence of root-knot nematodes and different stress conditions, such as drought and freezing temperatures.
3. The gradual withering back of older leaves, beginning at the tips, is one of the leaf symptoms. Galls develop on the primary and secondary roots, and after severe nematode infections, the roots can twist and occasionally split.
4. Because root-knot nematodes are nearly universally found in banana plantations (Daneel *et al.,* 2015), it is crucial to keep an eye on their population levels in immature crops. According to popular belief, these nematode pests can seriously harm young plants, resulting in suboptimal growth and production.

4. The spiral nematode, *Helicotylenchus multicinctus* (Cobb, 1893), Golden, 1956:

H. multicinctus and *H. dihystera*, two species of spiral nematodes, have frequently been associated with the banana crop. In bananas, nematodes can live as endo and ectoparasites. They are dispersed throughout all regions that cultivate bananas. In India, this nematode significantly reduces banana yields by up to 20%.

Life cycle:

1. In lesioned cortical tissues, an 8–26 egg group was observed.
2. In tap water at 30 degrees, it takes 48 to 51 hours for the eggs to hatch.
3. Within 36 hours after the inoculation, the adults and larvae penetrate the root's epidermis.
4. The life cycle takes 30 to 35 days to complete *(Jonathan*, 2001 *and* Nickle, 1984)

Signs and symptoms:

1. The symptoms that *H. multicinctus* causes above ground are comparable to those that other banana nematode pests induce. When this nematode pest's infection levels are extremely high, topping may happen (Gowen and Queneherve, 1990).

2. Blackening of the root epidermis, tiny reddish lesions in the superficial cortex, and a decrease in the number of lateral roots are signs of *H. multicinctus* injury (Jones and Milne, 1982).
3. Lesions consolidate and cause widespread necrosis in the outer root cortex and even root back when this spiral nematode is heavily infected.
4. The rhizome tissue of diseased plants may also have lesions, which causes plant dieback. The tissue of an infected plants rhizome may also include lesions.

5. The cyst nematode, *Heterodera oryzicola* Rao and Jayaprakash, 1978:

The key nematode found in bananas is the cyst nematode. The bunch weight might be reduced by 20.5–56.5% by an initial inoculum of 100–1000 live cysts per plant at planting time. The root weight is significantly reduced when these nematodes attack the thin lateral roots.

Life cycle:

1. These cyst nematodes that form cysts are sedentary in nature. The eggs are contained in cysts, which are long-lasting tanned sacs produced by the female body. Cysts can live in the soil for many years.
2. Second-stage juveniles (J2) break through host roots and form a specialised feeding location (syncytium) in the stele after emerging from the cysts.
3. From the second stage juveniles of one generation succeed in the second stage, as they grow, the females become bloated, retaining the eggs and producing huge egg masses.
4. The life cycle of *H. oryzicola* was completed in 30 days.

Signs and symptoms:

1. All species have symptoms that are identical. A smaller number of tillers are produced by infected plants, which are severely stunted and chlorotic.
2. There is a reduction in root growth, with the nematodes' feeding appearing to have spurred the appearance of many more tiny roots in the root systems.
3. On the roots, you can see brown cysts and white females with lemon-shaped markings.

6. Reniform nematode, *Rotylenchulus robutus* (de Man, 1876):

Rotylenchus sp. is not thought to be a common pest in the banana industry, its presence in bananas has not been thoroughly investigated.

Life cycle:

1. The temperature 13°C is not ideal for survival, although 18.5°C and 24°C are both suitable temperatures for growth.

2. Within the eggs, the first moult takes place, and after 14 to 16 days, the second-stage juvenile hatches.
3. Only after approximately 100 days at a temperature of 23°C does the second generation develop; the fourth larval stage occupies the majority of this time.
4. After the third moult, male and female juveniles can be distinguished from one another by the body lengths and light spots near the gonad that are still developing.

Signs and symptoms:

1. Syncytia forms close to the root pericycle.
2. Eggs deposited in a matrix of gelatin.
3. Above ground: stunting, leaf shedding, and dwarfing.
4. Root discolouration and necrotic (dead) regions with degradation are visible below ground.

Other nematodes:

Although many other nematode species have been associated with bananas, they are not taken into account commercially. The most common genera of Plant-parasitic nematodes associated with bananas are *Helicotylenchus, Tylenchorhynchus, Hoplolaimus, Xiphinema, Hirschmanniella, and Criconemoides* (Khan *et al*; 2004).

Nematode management:

Reduce the initial inoculation level and control their progressive expansion by using intercropping and proper cultural practices as effective preventative strategies. An essential preventative measure for nematode management is the use of planting materials free of worms. Appropriate cultural and chemical treatments are important to limit the losses brought on by nematodes. The use of chemicals to control nematodes on bananas is explored in numerous methods.

Chemical methods:

Sundararaju *et al*. (1999) show that Root-lesion nematode causes substantial decrease and yield loss in bananas, a field experiment was done on three commercial banana cultivars in heavily infested fields. This was accomplished by applying two nematicides, Monocrotophos and Carbofuran, at varied doses and times, coupled with the prescribed sucker paring and pralinage practises. The results showed that both compounds administered at different times were successful in lowering nematode populations and greatly enhancing plant growth and output when compared to the untreated control. Some treatments were found to be very effective in reducing nematode populations and significantly increasing the yield of Karpuravalli, Monthan, and Nendran (26.0 kg, 22.3 kg, and 9.9 kg, respectively) compared to untreated controls (16.2 kg, 15.1 kg, and 4.7 kg,

respectively). Sucker dip with Monocrotophos at 0.5%, Bavistin at 0.1%, followed by Carbofuran at 50 g/plant applied at 3 and 6 months after planting or application of Carbofuran at 50 g/plant once at the time of planting in the pit or dipping of the suckers with mud slurry and sprinkling with Carbofuran at 50 g/sucker and two applications after planting at 3 month intervals are among these.

Cultural methods:

It has been discovered that proper cropping sequence, summer ploughing, compost application in soil, and soil integration of green manuring crops are efficient in reducing the population of plant parasitic nematodes in soil. (Pranjal Pratim Neog 2020). Banana is a perennial crop; it is necessary to manage nematodes for a longer period of time after planting. Planting low-growing cover crops that produce nematode-allelopathic chemicals is an excellent choice. Allelopathy is a biological condition in which one or more biochemicals produced by one organism negatively affect the growth, survival, and reproduction of other organisms. Sunn hemp (*Crotalaria juncea*), marigold (*Tagetes* spp.), rapeseed (*Brassica napus*, Wang *et al.,* 2001), velvetbean (*Mucuna pruriens*, Zasada *et al.,* 2006), and sorghum-sudangrass (*Sorghum bicolor*, *Sorghum arundinaceum* var. Sudanense, Widmer and Abawi 2000). Because of its low-growing habit and the fact that it does not require soil integration to be allelopathic, marigold stands out as an especially excellent candidate for banana cropping systems. Marigold's allelopathic effect varies depending on the marigold and nematode type, cultivar, and soil temperature (Ploeg and Maris 1999). *Tagetes patula* 'Single Gold' consistently controlled a wide spectrum of plant-parasitic nematodes in field experiments. Only living marigold root systems have nematicidal qualities; incorporating 'Single Gold' wastes into the soil has little effect on root-knot nematode suppression (Ploeg 2000). Growing *T. patula* and *C. juncea* in plant-parasitic nematode-infested field soil dramatically reduced nematode population densities after 5 months when compared to ongoing banana growing in these soils in a greenhouse experiment (Koon-Hui Wang *et al.,* 2018).

Biological methods:

Endophytic fungi were isolated from banana accessions belonging to distinct chromosomal groups, and their bio-efficacy against P. coffeae and *M. incognita* was evaluated in vitro. *Fusarium* spp. was identified as the endophytic fungus isolated from bananas, and their biocidal effects on P. coffeae and *M. incognita* were investigated. In vitro, culture filtrates of endophytic fungi isolated from diploids had a greater nematicidal impact than those obtained from triploids. Endophytic fungi's nematicidal effects on *P. coffeae* and

M. incognita juveniles increased with increasing exposure time to culture filtrates (Sundararaju *et al.,* 2002). Chemical pesticides are being used less frequently due to rising organic agriculture demand and environmental concerns. Crop growers are very interested in alternative pest management methods, such as the use of biological treatments. The effectiveness of nematophagous bacteria and fungi in controlling certain nematode pests, such as cyst and root-knot nematodes, has been well documented (Kullnig-Gradinger *et al.,* 2002, Yedidia *et al.,*1999). *Pasteuria* spp. parasitic bacteria have been found to infect 323 nematode species, including both plant-parasitic and free-living nematodes (Sikora *et al.,*2000). For nematode control, three application strategies for *P. penetrans* were evaluated: seed, transplant, and post-plant treatments (Bennett *et al*.,2009).

Jesus *et al.* (2015) findings show that the sisal liquid residue has the potential to control *R. similis* and that the fermentation process does not inactivate this residue or the components responsible for the nematicidal impact. Nevertheless, the fresh residue is a better alternative for the control of *R. similis* in vitro because considerably greater mortality rates were seen with concentrations of 20 and 25 %. Several ruminant studies have shown that plant extracts affect the development and death of nematode juveniles (Alonso Diaz *et al.,* 2008) and adults (Hounzangbe Adote *et al.,* 2005). Silveira *et al.,* (2012) investigated the antiparasitic efficacy of a sisal liquid residue on the in vitro egg hatching and juvenile growth of goat gastrointestinal nematodes.

Integrated Nematode Management:

According to Vidya and Reddy (1998), the integration of neem cake (neem extracts), Carbofuran, *P. penetrans*, and *Glomus fasciculatum* was the most effective in enhancing banana plant growth and yield, raising the cost: benefit ratio to 1:2.65, and reducing *R. similis* populations in soil (95.16%) and roots (89.27%). According to Ravi *et al.,* (2000), incorporating 250 g neem cake, 20 g *T. viride*, and Carbofuran 3G at 10 g/sucker resulted in a reduction of *R. similis* population in soil and banana roots. Coyne *et al.,* (2010) found *Helicotylenchus multicinctus, Radopholus similis*, and *Meloidogyne* spp. to be 0.7% more abundant than controls. A method for removing nematode pests of banana and plantain (Musa spp.) by simply immersing the peeled or unpeeled suckers in boiling water for 20-30 seconds has proven to be more friendly to small-scale farmers and is superior to hot water treatment at 50°C because the time required to treat a sucker is reduced and temperature and timing measurement is simplified. It disinfects suckers of various sizes without impairing germination (Hauser and Coyne, 2010).

After two years of investigation, Roy *et al.,* (2016) discovered that paring + hot water treatment of suckers at 550 C for 10 minutes + carbofuran 3G @ 0.5gm / plant + neem cake@ 1 kg/plant (at the time of planting) produced the greatest results. Banana yield qualities (fingers/hand, hands/ bunch) and fruit production were increased by 30% over the untreated control, whereas soil populations of *Meloidogyne incognita, M. javanica, Rotylenchulus reniformis* and *Hoplolaimus indicus* were dramatically reduced. This treatment also had the lowest root-knot index due to Meloidogyne spp. infection.

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THE ROLE OF NANOPARTICLES IN ENHANCING PLANT TOLERANCE TO ABIOTIC STRESSORS

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Abstract:

A critical issue in agriculture, high salinity damages plants by limiting their ability to absorb water and by introducing too many ions into the transpiration stream, which damages plant cells. As a result, high salinity annually results in large production losses in crops. It has been demonstrated that applying diverse bioactive nanoparticles of specific concentrations, shapes, and sizes can protect different agricultural plants from salt stress while also enhancing growth metrics, seed germination, root morphology, yield, and metabolic profile. Because of their small size, enhanced surface area and absorption rate, catalysis of processes, and appropriate reactive sites, nanoparticles are being increasingly exploited as a tool to stimulate specific biochemical events relevant to plant eco-physiological output. In saline environments, controlled eco-physiological processes may be essential for promoting plant development and ensuring plants survive in lessthan-ideal conditions. Salinity is one of the environmental factors affecting crop yield and is one of the most common conditions in the world. Selenium is essential for human health due to its role as a cofactor for enzymes involved in antioxidant

Introduction:

Agricultural production, the ecology, and the economy are all negatively impacted by soil salinization. Plants under salinity stress may experience physiological and molecular alterations. While many plants are genetically salt-sensitive, certain plants have salttolerance genes. Under abiotic environments, a number of intricate mechanisms may modify the genetic responses of plants. According to the FAO report 2021, roughly 833 million hectares of soil (8.7% of the planet) are damaged by salt. The excessive salt concentration of crop field soil effects plant resilience and decreases crop yield. Many times, cyclones have caused severe salinization of crop fields. For example, cyclone Aila in 2009 severely damaged agricultural fields in the Sundarbans lay (West Bengal, India),

which led to a subsequent sharp decline in crop production (rice, legumes). Cyclones Fani in 2019, Amphan in 2020, and Yaas in 2021 have also severely degraded numerous croplands, primarily in West Bengal and Odisha, India. In more than 100 nations worldwide, soil salinity is increasing, primarily as a result of sea level rise, according to the Joint FAO/IAEA Report 2020.

The soil has become salt-affected as a result of factors like poor groundwater quality, sealevel rise, extended drought, irrigation, and improper chemical fertilizer or pesticide use, endangering global food and nutrition security as well as the livelihood of many farmers. This endangerment threatens the nation's economy. The agricultural field has reported effects of nanoparticles, focused on improving seed germination, plant growth, and photosynthetic rate. Because of their distinct physicochemical features that confer antibacterial and antioxidant capabilities, silver nanoparticles (AgNPs) exhibit markedly superior behavior above other nanoparticles. The family Capparaceae includes the rare medicinal plant *Maerua oblongifolia* (Forssk) A. Rich, which is sold in Saudi Arabia. It is frequently used in Saudi Arabia's traditional herbal medicine traditions to treat a variety of illnesses in both people and domestic animals. The salinity in Saudi Arabia, overuse for food, medicine, and other purposes, as well as this plant's slow rate of regeneration, are all contributing to the rapid decline of the wild populations of this plant. Since the saline environment has seriously impacted the distribution of *M. oblongifolia* plants, there is a major need to improve their growth and morphogenesis.

Chitosan is a non-toxic, biodegradable and biocompatible substance which reduces the adverse effects of abiotic stresses through the pressure transduction pathway via secondary signaling. Grape (*Vitis vinifera* L.) is a perennial plant in the family Vitaceae that is widely planted in the world and is one of the most economically important fruit crops. In agriculture, metalbased nanoparticles such as silver, copper, and zinc oxide have gained attention due to their antimicrobial and plant growth-promoting properties

The application of Zn NPs to salt-stressed Brassica napus plants attenuated the adverse effects caused by salinity by mechanisms of antioxidant up-regulation, osmolyte biosynthesis, and ion control. In Solanum lycopersicum, foliar application of Cu alleviated salinity stress by improving growth and Na+/K+ ratio. In addition, Cu NP improved levels of glutathione, polyphenols and vitamin C.

The use of nanoparticles on plants lessens the effects of salt stress. Nanoparticles can alter the genetic makeup of plants to make them resistant to salt stress. They are naturally occurring and can be found in a variety of materials, such as minerals or as a

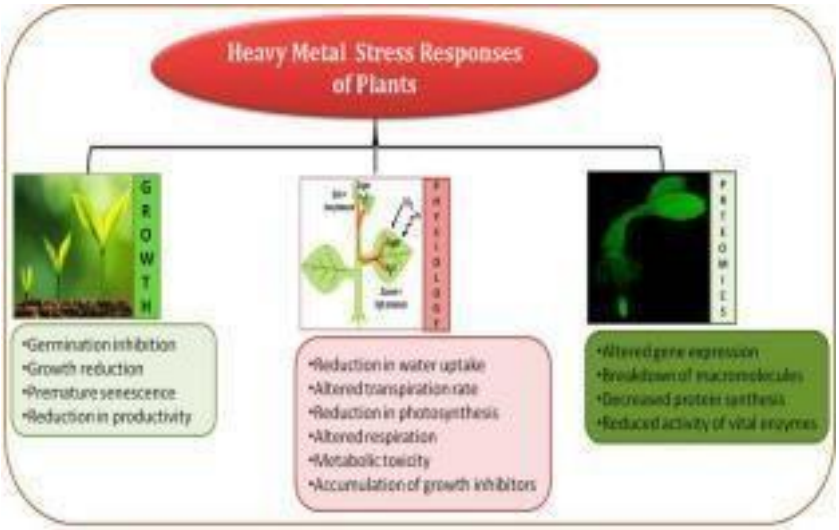
byproduct of bacteria and clays. Engineered nanoparticles have a few key unique characteristics that are important. These nanoparticles are very different from nanoparticles that naturally occur because they differ greatly in size, shape, and content. Nanoparticles have previously been used to color metals and for various purposes, but in recent years, new uses have emerged. Nanoparticles can be made to aggregate and oxidize in response to environmental conditions. Chemicals in the environment, as well as physical characteristics, can influence the stability and behavior of nanoparticles. The composition of nanoparticles determines their qualities. The composition of nanoparticles influences their pace of reaction, ability to penetrate, and transportation inside the plant.

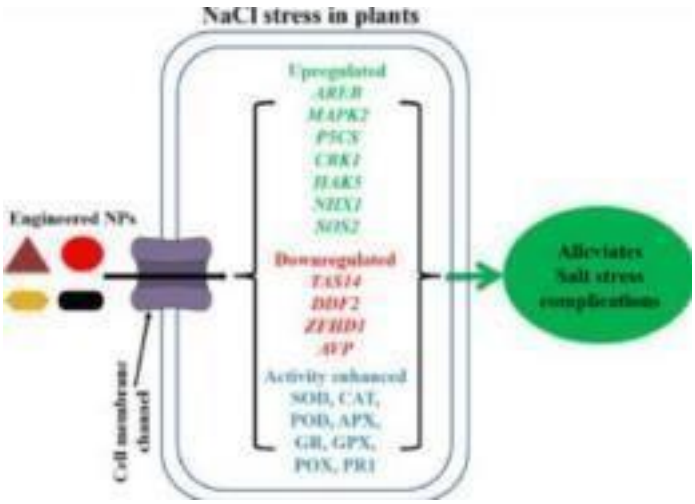
Currently, the" green" system of nanoparticle combination has come a subject of interest because conventional chemical styles are costly and bear the use of chemical composites organic solvents. reducing agent is also poisonous. Growth inhibition was set up to be particularly higher in biosynthetic ZnO than in chemical ZnO nanoparticles as well as other common antimicrobial agents. The increased bioactivity of these lower patches is due to the advanced surface- to- volume rate. Salt stress causes the generation of excessive reactive oxygen species (ROS), In this way, the negative effects of oxidative stress brought on by high salt stress are reduced. Further research is needed to examine the intricate molecular pathways behind the interaction between the NPs and the genes or enzymes. Due to its special qualities and wide range of uses in transparent electronics, UV light emitters, piezoelectric devices, chemical sensors, and spin electronics, ZnO

NPs have gained a lot of research attention. ZnO is non-toxic and can be used to photocatalyze the breakdown of contaminants in the environment. ZnO has showed remarkable sensitivity for a variety of hazardous gases in both bulk and thin films. Different zinc oxide nanoparticle concentrations were applied to peanut seeds. In order to improve seed germination, seedling vigor, and plant growth, zinc oxide nanoscale treatment (25 nm mean particle size) at 1000 ppm concentration was applied. These zinc oxide nanoparticles also demonstrated effectiveness in boosting stem and root growth in peanuts.

NPs enter a plant's system in a number of ways, mostly through the roots and leaves. After entering the plant, NPs interact with it at the cellular and subcellular levels, leading to changes in morphological, biochemical, and physiological molecular states. Depending on the NPs and the plant species, these interactions could be advantageous or harmful. Effects of NPs on plant systems may depend on the chemical make-up, reactivity, size, and particularly concentration of the particles within or on the plant. Various NPs can

stimulate plant growth and development in salinity-stressed environments at concentrations below specific limitations by a variety of known processes, according to the evidence currently available. The majority of these investigations were carried out in pots or plate growth media, which are artificial treatment settings. We explore the benefits of NPs to increase plant tolerance to salinity stress in order to comprehend how they affect plant growth.





Chlorophyll fluorescence parameters

Saline stress and foliar fertilization as well as NC CS-SA on photosynthetic active radiation (PAR) values showed that foliar fertilization in the 0 and 50 mM salinity treatments did not significantly affect to the value of administrative reform. The highest PAR values were observed at 0.5 mM NC CS-SA under 100 mM salinity.

According to the results, N content decreased significantly with increasing salinity, while foliar application of CS-SA NC fertilizer improved this content at different salinities. P content decreased significantly with increasing salinity pressure and the maximum

concentration belonged to 0.5 mM CS-SA NC treatment without salinity; 100 mM NaCl without foliar fertilization contained the lowest P content. Proline plays a fundamental part as an osmotic stabilizer as well as a stabilizer and defender of chemicals, proteins, and films. Application of chitosan in chickpeas increases K content under salinity pressure70. It seems that the administration of chitosan induced an appropriate response of saline vines to salinity stress by increasing and decreasing K and Na concentrations, respectively; in other words, chitosan can minimize the ionic toxicity caused by salt stress.

Molecular insights

Abscisic acid (ABA) has been characterized as a stress hormone, accumulating in response to stress and mediating many stress responses for plants to adapt to climatic conditions. It is reported to be involved in water-restricted environments possibly as a result of drought and salinity stress. Several studies have demonstrated an important role of the SOS pathway in maintaining ionic homeostasis at the cellular level and salt tolerance. Application of nano ZnO (100 mg/L) in Z. mays enhanced melatonin synthesis and activated antioxidant enzymes, alleviating the effects of drought. Compared to the control, Cd addition significantly reduced root, stem and leaf biomass at the seedling stage. Simultaneous application of Cd and

Nano-Se significantly improved the biomass of hot pepper tissue compared to Cd treatment. Cd1Se0.2 and Cd1Se1 increased root (38% and 30%, respectively), stem (33% and 26%, respectively), and leaf (30% and 29%, respectively) biomass compared to Cd treatment.

The effects of copper nanoparticles on maize plants. They found that exposure to copper nanoparticles led to a decrease in plant height, chlorophyll content, and root biomass. The plants also exhibited signs of oxidative stress, indicating potential toxicity of copper nanoparticles to agricultural crops. Silver nanoparticles led to reduced seed germination, altered root morphology, and decreased photosynthetic efficiency. The study highlighted the potential risks associated with the use of silver nanoparticles in agricultural practices.

Microalgae, single-celled prokaryotes or aquatic dominant eukaryotes that perform photosynthesis form colonies without any cellular differentiation and can grow in a variety of media such as freshwater, saltwater and marine. Microalgae have been widely used in many industrial, medical and biotechnological applications through many potential biological applications, such as pigment overexpression, bioremediation, natural biological data and toxicity studies. The nanoencapsulation subsection casts a spotlight on

nanoencapsulation's transformative role in nutrient delivery. Expounding on the diverse encapsulation strategies – from polymeric to lipid-based carriers – it elucidates how these systems target nutrient release to match plant requirements, fostering efficient nutrient utilization.

CNPs

In addition to metal nanoparticles, carbon nanomaterials are classified into several forms such as nanotubes, graphene and fullerenes. However, the effect of carbon nanomaterials depends on the size, concentration and solubility of the applied nanomaterial, leading to opposite effects on seed germination.

To test whether the improvement in seed germination under saline conditions was primarily due to water uptake during CNP pretreatment, lettuce seeds were treated with HO, 150 mM NaCl, 250 mM NaCl, CNP, CNP plus 150 mM NaCl, and pretreated with CNP plus 250 mM NaCl for 4 h. Incubate them in 150 NaCl solution for 9 days. After 4 h of HO pretreatment, the seeds of Lg, Br, and Je were still barely germinated, while the seeds of Pi, Bc, and Mu were able to germinate about 35%.

Together, these results indicate that improved seed germination by CNP treatment is not simply due to water absorption during pretreatment and that pretreatment with saline solution does not improved seed germination of the lettuce varieties tested. Reports of NP-induced root growth inhibition vary widely from nanoparticle to nanoparticle and from plant to plant. For example, CNP stimulated root growth in cucumber (*Cucumis sativus*) and onion (*Allium cepa*), but inhibited root elongation in tomato (*Solanum lycopersicum*) and lettuce, suggesting that the response to nanomaterials can be species or genotype dependent.

Exposure to iron nanoparticles led to a significant increase in oxidative stress markers, such as lipid peroxidation and hydrogen peroxide levels. The study demonstrated the potential toxicity of iron nanoparticles to crop plants.

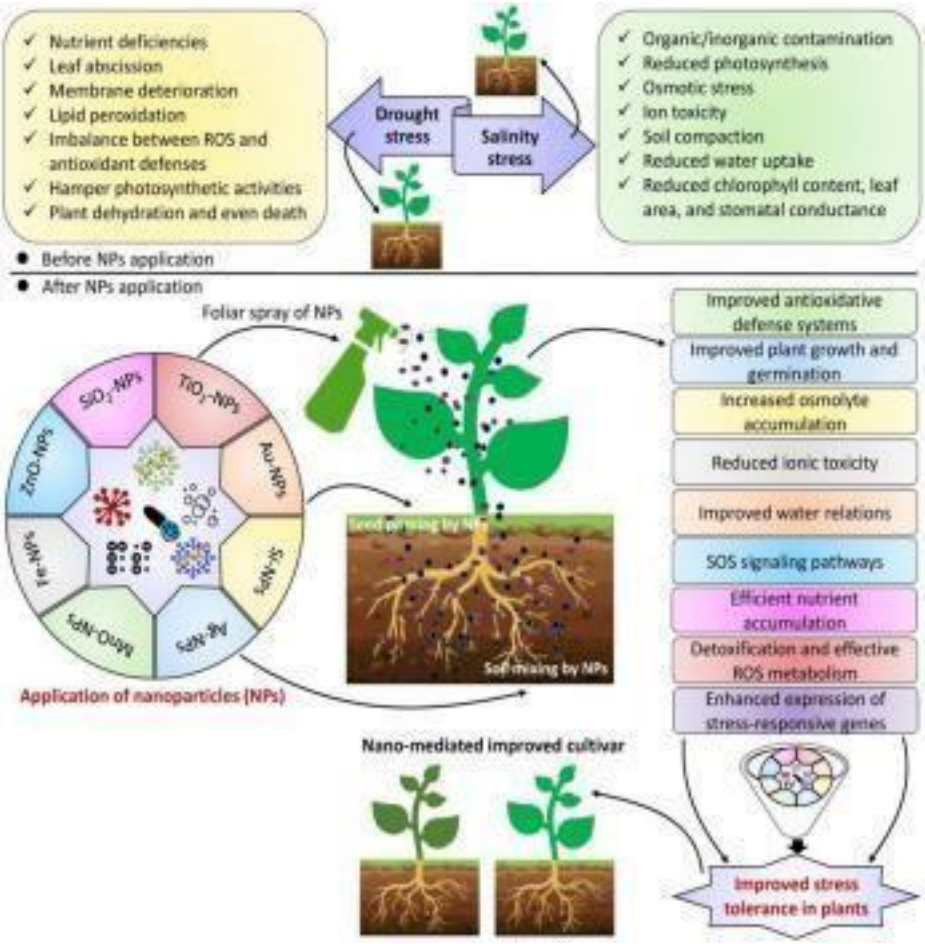
The six most susceptible cultivars were identified belonging to different types (Leaf, Avocado Head, Romaine), indicating salt stress susceptibility not specific to a given species. Primer or seed pretreatment with nanoparticles has been shown to promote seed germination of various crops. PAL is of great importance as it participates in phenol biosynthesis by creating a trans-cinnamic acid scaffold from phenylalanine. Therefore, the activity of this enzyme can affect the activity of other non-enzymatic compounds. It has been repeatedly reported that applying different types of NPs can increase the content of bioactive compounds in fruits, as observed in this study.

Due to the economic value and high market demand of bell peppers, there is great interest in increasing the production of this crop. However, like other crops, it is constantly threatened by various abiotic stresses such as soil and water salinity. This stress-related problem is one of the major constraints in food production, especially in semi-arid and arid regions where the problem is most prevalent. In addition, NPs have beneficial effects on several chloroplast enzymes involved in the biosynthesis of photosynthetic pigments. This can lead to increased chlorophyll and other accessory pigments in plant leaves. This is particularly important because auxiliary pigments such as carotenoids are important antioxidants, and especially under stress conditions he is an important means of dissipating excess excitation energy of the PSII antennae.

The observed decrease in the pH of tomato fruit may be due to the accumulation of organic acids in the vacuole. This is consistent with Pinedo-Guerrero *et al.,* , who reported that application of Cu NPs + chitosan in jalapeños reduced the pH of the fruit. However, application of Cu NPs in tomato plants has been shown to increase the pH in the fruit.

Mechanisms of the salt tolerance in agricultural plants mediated by NPs

Although the precise mechanisms underlying the phenomenon of salt stress tolerance in plants by NPs remain obscure, it is known that certain NPs with particular characteristics are the agents responsible for upregulating or downregulating specific genes that are either directly or indirectly related to salt stress physiology. A brief illustration of the actions of the designed NPs on the regulation of various genes or antioxidant enzymes is provided. The specific overexpression of NHX1, a Na+/H+ antiporter gene, has been evaluated in several transgenic crops and has been shown to improve plant health by increasing the sequestration of excess ions caused by salt stress into the vacuoles. CeO_2 NPs have also been found to be active in upregulating NHX1 expression under salt stress in Arabidopsis thaliana and in rice, indicating that NPs can also reduce the effects of salt stress. Similar to this, when exposed to high salt concentrations, Ag NPs and CeO_2 NPs caused the SOS_2 gene, which is involved in the SOS (salt excessively sensitive) pathway of maintaining ion homeostasis and salt tolerance, to become upregulated, reducing salt stress. Several NPs, including SiO_2, ZnO, TiO_2, K_2SO_4, Cu, and Se, are found to be very active in significantly enhancing the activity of several antioxidant enzymes, including SOD (superoxide dismutase), CAT (catalase), POD (peroxidase), APX (ascorbate peroxidase), and POX (poxidase).



Because an imbalance in ionic and osmotic equilibrium impacts the oxidative system, homeostasis, and nutrient availability, minimizing salt stress eventually lowers agricultural output, it is important to take note of this issue. Contrarily, production areas are expanding in salt-affected areas as a result of intense pressure to achieve food security goals and meet the demands of the continuously expanding human population. By providing plants with nanoparticles, one can significantly reduce the negative impacts brought on by various severe environments, such as salt stress, and so control plant adaptation mechanisms. Agricultural soils worldwide are suffering from salt stress, and scientists are attempting to assess the usefulness of silver nanoparticles for growth under salt stress. Salt stress reduced crop production and significantly impacted crop growth on 125 million hectares.

Despite the fact that ZnO NPs are very important commercially and are used in many goods, there is an increasing public interest in learning about the toxicological and environmental impacts of ZnO NPs. Unfortunately, toxicological research on zinc oxide nanoparticles conducted in the past ten years indicates that ZnO NPs may pose dangers to both human health and the environment.

Mice, bacteria, Daphnia magna, freshwater microalga, and even human cells are all very hazardous to ZnO NPs.

Key findings from recent studies

When plants under salt stress were exposed to silver nanoparticles, their potassium and sodium chloride potassium levels improved. It has been discovered that silver nanoparticles are far more stable in low salinity fluids, and that continuous exposure to them can be utilized to treat plants injured by fluctuating salinity environments. The production of several transgenic plants is currently underway as researchers work to assure plant propagation in the field. Before planting, seeds are treated with nanoparticles as a way to help plants in field circumstances. Additionally, silver nanoparticles improve wheat grain germination. Reactive oxygen species (ROS) are also created in plant cell organelles such the plasma membrane, peroxisomes, chloroplasts, and mitochondria under both natural and stress settings in the tobacco plant, which highlights the significance of silver nanoparticles in accelerating seed germination. Reactive oxygen species (ROS) overproduction harms genotypes, growth, development, and increases stress in plants. Thiobarbituric acid reactive substances (TBARS) and hydrogen peroxide production were both reduced by the treatment of plants with AgNPs and NaCl. It has been shown that plants' capacity for antioxidant defense increases in response to the negative impacts of salt stress. It is known that a number of enzymes, including ascorbic peroxide, betaine, anthocyanin, catalase, dehydroascorbate reductase, glycine, glutathione reductase, monodehydroascorbate reductase, proline, and superoxide dismutase, protect plants from oxidative stress. In the past, it has been demonstrated that Ag nanoparticles derived from plants can activate plants' antioxidant defense mechanisms, reducing stress. Because salinity has a negative impact on agricultural productivity and growth, there is significant devastation.

More than 1 million hectares of agricultural land are lost to salinization each year as a result of this situation, which affects an area of more than 1000 million hectares, of which 76 million are occupied by humankind. To lessen the negative impacts of these pressures, fresh strategies are ultimately required. The osmolality, chloride, sodium, potassium, and chloride of salt concentrations in plants treated to AgNPs all considerably increased. By adjusting the salinity of aquatic environments, silver nanoparticle consistency can be controlled. It has been observed that these NPs are more consistent in less salinized water. Increased salinity may be detrimental to the development and output of plants. Certain cell structures, including mitochondria, chloroplasts, peroxisomes, and cell membranes,

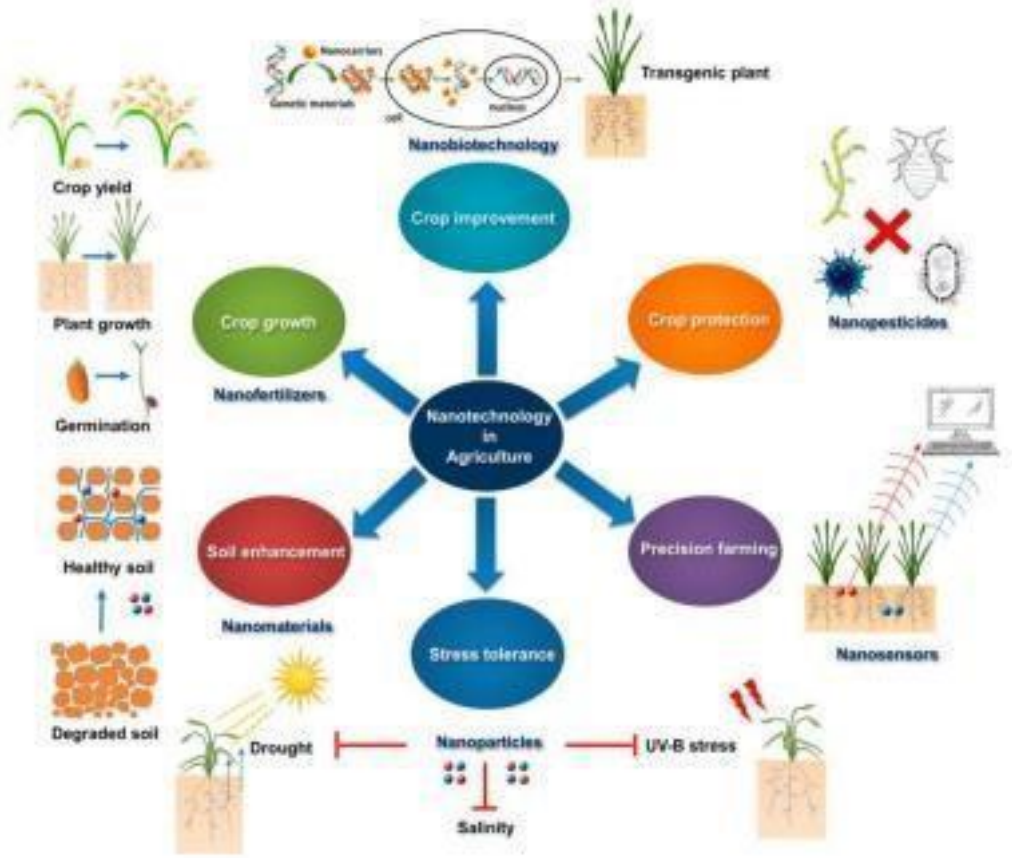
generate ROS both under normal conditions and under stressful circumstances. ROS overproduction in plants is related to oxidative disruption and influenced by salinity, genetic make-up, and stage of development. The combined sodium chloride and silver nanoparticles reduce H2O2, TBARS, and electrolyte leakage when compared to samples treated with sodium chloride. Because of the negative effects of salinity, plants develop stronger antioxidant defenses. Many antioxidant enzymes, including anthocyanin, catalase, ascorbate peroxidase, dehydro-ascorbate reductase, glycine, and glutathione reductase, and superoxide dismutase, play important roles in defense mechanisms. Previous studies have demonstrated that silver nanoparticles can reduce saltiness by activating antioxidant processes. In general, the work explained and stressed advantageous ways in Ag-nanomaterials that interacted with salinity tolerance, emphasizing that the methods of convincing tolerance are dependent on the antioxidant defense mechanism, ion buildup, and proline metabolism.

India produces a total of 500 million tonnes (Mt) of agricultural by-products annually. The use of agricultural waste to produce value-added products such as biochar, organic fertilizers, nanoparticles, biogas and pulp can significantly reduce the problem of agricultural waste management.

Chlorophyll, sugar, lipids, and anthocyanin carotenoids are all impacted by the salt of the environment. High salt stress has led to the evolution of many anti-oxidative enzymes that plants use to combat the stress, including CAT (catalase), SOD (superoxide dismutase), and POD (peroxidase), which are thought to be crucial in the detoxification of ROS (reactive oxygen species). Together, CAT (catalase) and POD (peroxidase) play a role in the breakdown of H_2O_2 into O_2 and H_2O. Proline, glycine betaine, polyols, and trehalose particle function are examined.

Meticulous characterization of nanoparticles and the assessment of their toxicological effects should be performed before being introduced into the agricultural system. Implementing regulations and guidelines for the safe use of nanoparticles can help minimize their potential negative impacts. Employing targeted delivery systems, encapsulation techniques, or modifying nanoparticle surface properties can reduce metal exposure and enhance their efficiency. Continuous monitoring of nanoparticle release, accumulation, and distribution in various compartments of agricultural ecosystems is essential to prevent uncontrolled metal stress. The effects of zinc oxide nanoparticles on soybean plants. The results showed that high concentrations of zinc nanoparticles caused

oxidative stress and reduced plant growth. It concluded that the use of zinc nanoparticles in agriculture should be carefully regulated to prevent adverse effects.



Proline helps plants overcome osmotic stress when the environment is salty. In Triticum aestivum (Poaceae), different concentrations of silver nanoparticles boosted fresh and dry weight, soluble sugar, total chlorophyll content, and antioxidant enzymes during salt stress. Application of six silver nanoparticle impacts on Plant 122 of 0–10 mg AgNPs increased seed sprouting, root and shoot length, and proline content under salt stress. Lathyrus sativus is a member of the Fabaceae family. After exogenous administration of 0–10 mg AgNPs under salt stress, Thymus vulgaris andT. Daenensis (Lamiaceae) showed bettered germination chance, seed vigor, and root and shoot length. also, varied AgNP attention enhanced fruit periphery and weight, branch count, factory height, and raying viscosity. The chance of germination, the length of the rootlets, and the fresh weight under swab stress were each bettered by about 20 mg/ kg AgNPs. At different NaCl concentrations (5, 10, 15, and 20 dS/m), low quantities of silver nanoparticles increased the germination rate. The application of compost and nano maghemite increased plant growth, while reducing soil availability of Cd and Zn as well as their uptake by sunflower compared with control treatments and using only compost (Martinez Fernandez *et al.,* 2015). Similarly, the combined application of SiO2 NPs, zeolite and biological supplements

in Zn-contaminated soil increased sunflower biomass while Zn concentrations in shoots and roots decreased significantly, as well as only Soil bioavailable forms of Zn have been observed (Mousavi *et al.,* 2018).

Furthermore, thousands of ENP-containing products are synthesized annually around the world (Kumar *et al.,* 2019). Although advanced experimental techniques can help us determine the behaviour and biological effects of these ENPs, it is difficult to cover all individual ENP types due to their high cost of money, time, and money. time and manpower. To date, inorganic ENPs (e.g., metal-based, carbon-based) have been explored more than organic ENPs (e.g. polymers and derivatives of biomolecules) (Rai *et al.,* 2018). A variety of environmental factors, such as pH, electrolytes, and dissolved organic matter, can influence the fate and activity of ENPs, which subsequently alter uptake, accumulation, and response. response of an organism to exposure to ENP in the environment.

Transport

Besides the stomatal pore size and the leaf cuticle, the adhesion ability of the nanoparticles on the leaves is another factor that can affect the efficient delivery of nanoparticles on the leaves in plants. Large NPs are always more easily washed out than small NPs. After passing through the epidermis of leaves through stomata or the cuticle pathway, leaf NPs are mainly distributed in plants by transport through the phloem. After crossing the leaf-epidermal barrier, NPs can reach the phloem by two main routes: 1) from mesenchymal cells (barrier and spongy mesenchymal cells) to the phloem, and 2) directly across the intercellular spaces to the libe. For pathway 1, the plasmodesmata between mesenchymal and bundle sheath cells (Danila *et al.,* 2016) could be the major pathway that allows transport of NPs from mesenchymal to bundle sheath cells, following there comes libe. For pathway 2, delivery of NPs through the cell wall of sheath cells may be considered to reach the phloem.

Different organs of tomato plants showed large differences in mineral content, excluding Ca and Fe. Higher levels of phosphorus, calcium, magnesium, sulfide, sodium, iron, copper and manganese were detected in leaves. On the other hand, K and Zn were higher in fruits. P, Mg, S, Na and Zn contents in leaves increased under salinity stress (5.3%, 5.0%, 4.6%, 166%, and 12.3%), K (11.8%), Fe (2.7%) and Mn (2.9%) contents. The content found in the current study may alter membrane selectivity and thereby modify membranes, regulates plant nutritional balance, leading to an increase in Na+/K. + interlocking ratio salt tolerance. Treatment of salt stress has been shown to strengthen this association. Tomato plants are experiencing impairments in stress tolerance.

ROS are normally formed as a by-product of plant cell metabolism. Various environmental stresses can lead to excessive production of ROS in plants Causes progressive oxidative damage. After exposure to Al2O3 NPs, N. arvensis root H2O2 content (a product of the superoxide dismutase reaction) increased at 50 and 100 mg/L, but was comparable in control plants at 1000 and 2500 mg/L. level has been greatly reduced. , indicating that an excessive amount of ROS was removed via high concentrations of antioxidant enzymes.

Conclusion:

In conclusion, the interplay of nanotechnology and agriculture holds transformative power. From redefining nutrient delivery to reimagining pest control, nanoparticles sculpt the contours of modern agriculture. As we navigate challenges and seize opportunities, the pursuit of nanoparticle-driven sustainable agriculture beckons, illuminated by the promise of a nourished planet and prosperous future. While the potential benefits are promising, it is crucial to acknowledge the need for continued research into nanoparticle safety, ecological impacts, and long-term efficacy. Ethical and regulatory aspects also require careful consideration. The field of nanoparticle-plant interactions continues to evolve rapidly. Future research should focus on optimizing nanoparticle formulations, dosages, and delivery methods.

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IMPACT OF PESTICIDES ON THE ENVIRONMENT

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Introduction:

Pesticides are compounds used to prevent or get rid of pests including insects, weeds, fungi, and rodents that can damage crops, animals, or humans. They can be found in a variety of forms, such as rodenticides, fungicides, insecticides, and herbicides. A pesticide should ideally be fatal to the pests it is intended to control but not to other species, including humans. Unfortunately, this is not the case, and the issue of pesticide misuse is gradually coming to surface. The widespread usage of these chemicals under the saying, "if little is good, a lot more will be better" has disastrously impacted humans and other life forms (Aktar *et al.,* 2009; Bondareva & Fedorova, 2021).

Without a corresponding rise in food production, the growth in the global population throughout the 20th century would not have been possible. Crop losses are estimated to be between 30 and 40 percent prior to harvest, 10 to 30 percent post-harvest, and 50 percent due to insects, weeds, diseases, and rodents. The use of pesticides is thought to be the sole way to protect agricultural products from pest devastation. This is why Pesticide consumption has grown over the past 20 years (Tudi *et al.,* 2021)**.** Pesticide use influences the production of almost one-third of agricultural products. The production of fruits would decrease by 78%, vegetable production by 54%, and cereal production by 32% without the use of pesticides. Therefore, pesticides are essential for decreasing illnesses and raising crop yields all around the world (Ahmed *et al.,* 2016).

Five thousand years ago, sulfur was employed as a pesticide to eradicate insects and mites. The ancient Greeks and Romans employed oil, ash, sulfur, and other substances to protect their livestock and crops against various pests, while the Chinese used compounds containing mercury and arsenic to get rid of body lice and other pests. Crop rotation, tillage, and manipulating sowing dates were only a few of the cultural pest control techniques used. Around the time of World War II, the synthetic organic chemical industry started to grow, introducing the modern era of chemical pest control. Organochlorines,

such as dichlorodiphenyltrichloroethane (DDT), were the first synthetic organic insecticides to be produced (Ahmed *et al.,* 2011).

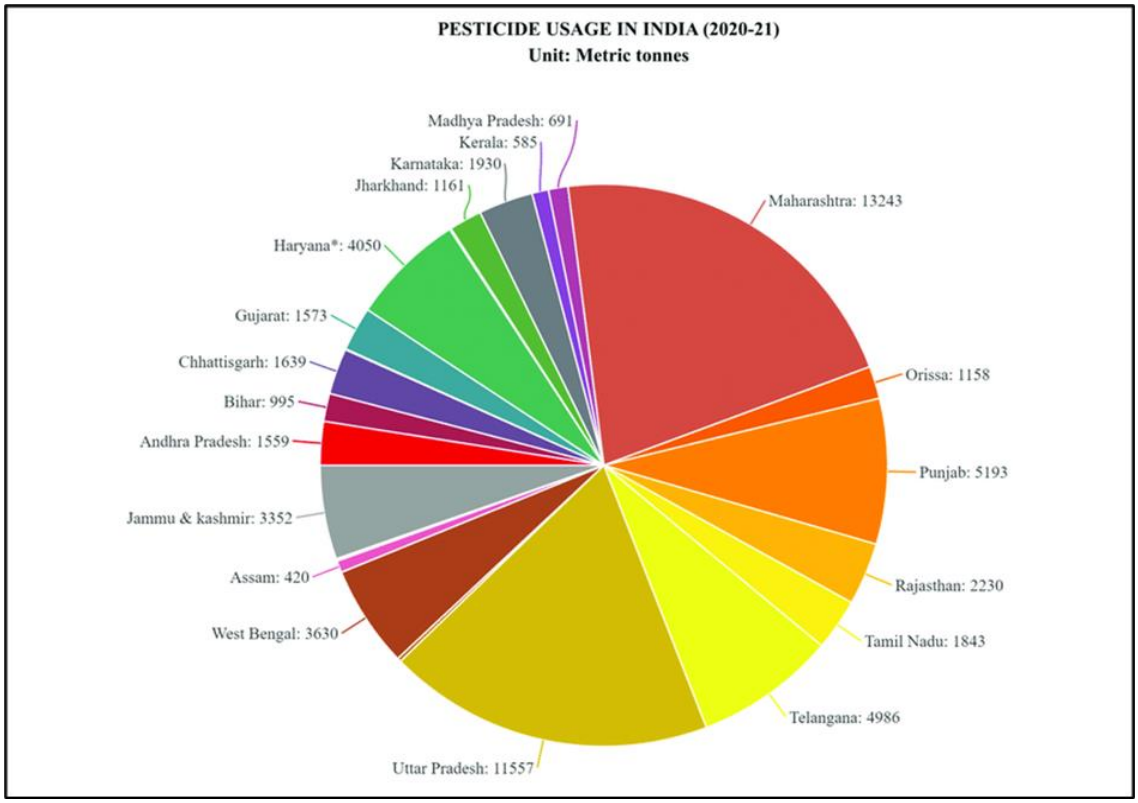

Figure 1: Pesticide use in different states of India (2020-21) (Raj *et al.,* 2021)

On a global scale, 50,000 species of plant pathogens, 9000 species of insects and mites, and 8000 species of pest plants are responsible for crop losses. In terms of crop loss, plant diseases, pest insects, and pest plants are all responsible for approximately 13%, 14%, and 13% of the harm caused to crops, respectively. Pesticides are also necessary for growing plants, especially commercially significant crop**s.** Plant diseases, weeds, and pests collectively cause the loss of 40% of agricultural produce worldwide. Crop losses would have been significantly higher in the absence of pesticides. Additionally, these crop-saving agents not only protect crops from pest damage but also significantly boost crop yields. Crop production has increased significantly as a result of the use of pesticides, and crop production would decrease if crops were not protected from the destructive effects of pests (Pathak *et al.,* 2022).

Types of pesticides:

To control pests and safeguard plants, a variety of pesticides are utilized. These pesticides can be classified according to the organisms they attack and their chemical composition.

1. **Insecticides:** These pesticides are made to control and get rid of insects that can damage crops. Aphids, caterpillars, beetles, and other hazardous insects are their main targets. A neonicotinoid insecticide called imidacloprid is frequently used to manage sucking insects like aphids, thrips, and whiteflies. chlorpyrifos is used to manage a variety of pests in crops like fruits, vegetables, and cereals. Malathion is an insecticide used in urban and agricultural settings to control flies, mosquitoes, and other insects. Permethrin is a synthetic pyrethroid pesticide that is used to manage a range of pests, such as fleas, ticks, and mosquitoes.
2. **Herbicides**: Weeds that compete with crops for nutrients, water, and sunlight are controlled with herbicides. They increase crop production and aid in controlling weeds in the fields. They may exert their effects by impeding particular plant growth and metabolism-related enzymes, interfering with the synthesis of vital plant hormones, or compromising the integrity of cell membranes. As a result, the targeted weeds experience limited development, wither, and eventually die. for instance, A broad-spectrum herbicide called glyphosate is used to manage weeds in a variety of crop and non-crop environments. The herbicide atrazine is frequently used to control weeds in maize and other crops. The chemicals 2,4-D, Clethodim, Glyphosate, Bentazon, and Clethodim are examples of herbicides used in agriculture (Jacquet *et al.,* 2022a).
3. **Fungicides**: In order to prevent or control fungal infections that can impact plants, fungicides are used, specifically targeting fungi. They are extremely beneficial in protecting against rust, mildew, and blight diseases. Fungicides fight fungi that cause plant diseases. They may work by impairing the development of fungal cell membranes, obstructing cellular respiration, or obstructing a particular enzyme's ability to carry out a specific activity required for fungal development and reproduction.
4. **Rodenticides**: Rodents like rats and mice can harm crops and spread disease, hence, rodenticides are used to control them. They either kill rodents by inducing internal bleeding, disrupting blood clotting mechanisms, or damaging their neurological systems. Some examples of rodenticides are chlorophacinone, diphacinone, brodifacoum, and bromadiolone.
5. **Nematicides:** Nematodes are microscopic worms that may severely damage plant roots. Nematicides are specific pesticides used to control them. Nematicides aim to kill or severely restrict plant parasitic nematodes. They can damage nematode cell

membranes, prevent the actions of enzymes, or obstruct nematode motility and reproduction.

Other than the above types, molluscicides are used to control mollusks like snails and slugs, which can harm plants, particularly in moist settings; acaricides take aim at mites and ticks that can harm crops and spread disease; and avicides are used to control birds that can harm crops or endanger certain environments.

Benefits of insecticides:

1. **Increased crop yields:** Pesticides help protect crops from pests, diseases, and weeds, which can lead to higher agricultural productivity and increased food production. This increased productivity may contribute to food security by ensuring stable and sufficient food supply (Bonmatin *et al.,* 2015).
2. **Disease Prevention:** Pesticides can help prevent the spread of diseases carried by insects and rodents, which can have significant impacts on human and animal health. Pesticides help control disease-carrying pests like mosquitoes, ticks, and rodents that can transmit diseases such as malaria, Zika virus, and Lyme disease to humans.
3. **Reduced Losses:** Pesticides can minimize post-harvest losses due to pests, spoilage, and contamination, thereby reducing food waste and improving overall efficiency in the food supply chain.
4. **Cost Savings:** Effective pest control through pesticides can reduce the need for manual labor and other resource-intensive methods of pest management, potentially saving farmers time and money.
5. **Biodiversity Conservation:** Pesticides can be used to control invasive species that threaten native plant and animal populations, helping to preserve local ecosystems.
6. **Aesthetic and Recreational Benefits:** Pesticides can be used to maintain the appearance and health of landscapes, gardens, and recreational spaces by controlling weeds and pests that detract from their appeal.
7. **Increased availability of affordable food:** Pesticides can help stabilize food prices by preventing crop losses and maintaining an adequate supply of produce. In forestry, public health, home settings, and, of course, in agriculture, which is a key component of the Indian economy, pesticide use has resulted in enormous benefits (Pathak *et al.,* 2022).

Negative impacts of pesticide use:

Pesticides are designed to target specific pests, but they can also harm non-target organisms, such as beneficial insects, birds, fish, and mammals. This can disrupt the balance of ecosystems and lead to declines in populations of important species. Pesticides can leach into groundwater or runoff into nearby rivers, lakes, and streams. This pollution can contaminate water sources, affecting aquatic life and potentially harming human health if these water sources are used for drinking or irrigation (Kumar *et al.,* 2012). Pesticide residues can accumulate in the environment, including in soil, water, and plants. Over time, these residues can build up and persist, posing long-term risks to ecosystems and potentially entering the food chain. Wind can carry pesticide particles beyond their intended target areas, leading to unintended exposure for nearby crops, wildlife, and even human populations. Pesticide drift can result in direct harm or contamination of non-target areas (Pathak *et al.,* 2022).

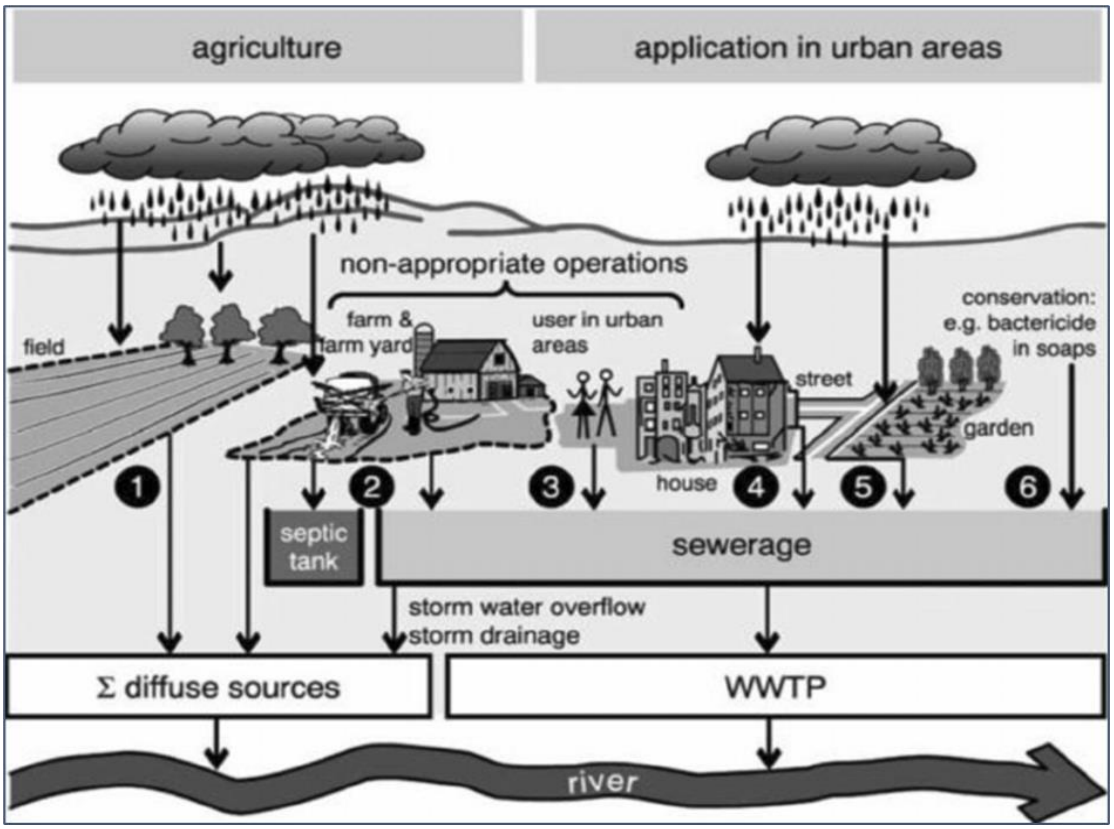

Figure 2: Major pathways from which pesticides can be transported into surface waters (Bonmatin *et al.,* 2015)

Over time, some pests can develop resistance to the pesticides used against them. This can lead to a cycle of increasing pesticide application and more potent chemicals, which can harm the environment and increase costs for farmers. Pesticide use can reduce

biodiversity by harming or killing non-target organisms, including pollinators like bees and butterflies, which are critical for the reproduction of many crops and the health of ecosystems. Pesticide-related declines in pollinators, natural predators, and soil organisms can disrupt important ecosystem services such as pollination, biological pest control, and nutrient cycling. Pesticides can impact soil health by reducing the populations of beneficial microorganisms and affecting soil structure (Sharma *et al.,* 2019). This can lead to decreased soil fertility and water-holding capacity, which in turn affects plant growth. Pesticides can potentially pose risks to human health, especially for those working in agriculture or living near treated areas. Exposure to pesticides has been linked to various health issues, including respiratory problems, developmental disorders, and certain types of cancer Ultimately, the benefits of pesticides need to be carefully weighed against their potential risks, and integrated pest management approaches that combine various strategies should be considered to minimize negative impacts while effectively managing pest populations (Mahmood *et al.,* 2016)**.**

Ways to reduce the use of pesticides:

Reducing the use of pesticides is a vital step towards fostering a more sustainable and environmentally conscious approach to agriculture. This can be achieved through the adoption of integrated pest management (IPM) practices, which emphasize a multifaceted approach to pest control. By implementing crop rotation and diversification, farmers disrupt pest life cycles and create less favorable conditions for their proliferation. Additionally, encouraging the introduction of natural predators and utilizing resistant crop varieties helps to maintain a balanced ecosystem without relying heavily on chemical interventions. Embracing precision agriculture techniques, such as targeted application based on data analysis, further minimizes pesticide use by ensuring that these chemicals are utilized only where necessary. Empowering farmers with education, promoting organic farming methods, and fostering consumer awareness also contribute to the collective effort of reducing pesticide use while safeguarding crop yields and environmental well-being (Jacquet *et al.,* 2022b).

Conclusion:

In conclusion, the impact of pesticides on crops is a complex and multifaceted issue. While pesticides can effectively control pests and increase crop yields, their indiscriminate use can lead to environmental and health concerns. It's crucial to strike a balance between pest management and sustainable agricultural practices to ensure food security while

minimizing adverse effects on ecosystems and human well-being. Continued research and the adoption of integrated pest management strategies are essential for a more resilient and harmonious coexistence between agriculture and the environment.

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PRODUCTION OF SINGLE CELL PROTEIN (SCP) USING AGRICULTURE WASTE

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Introduction:

Most often, proteins are referred to as the building blocks of all living things. Proteins are essential for the growth and development of living things and operate as enzymes to carry out a variety of different biochemical processes. Unlike other macromolecules, proteins are far more abundantly needed by our body. The post-modern era has given way to the modern era, but global poverty persists.

According to data gathered by the UN food and agricultural organization, 25% of the world's population lacks enough protein, which is a concerning sign of a protein gap. Traditional animal husbandry, however, is unable to provide the existing population with the necessary amount of protein-rich food. Therefore, a substitute protein source was created to satisfy the needs of the contemporary population. SCP (Single Cell Protein), healthy edible solitary celled bacteria, were created for this purpose. Carol Wilson originally coined the term "single cell protein" in 1967 to replace the less appealing terms "petro-protein" and "microbial protein." Since most of the microorganisms used are unicellular, their protein is referred to as "single cell protein." the biomass or significant proteins isolated from pure or different lineages.

In order to meet the world's growing population's nutritional needs, it is imperative to concentrate on novel, alternative, and unconventional protein production. SCP Plant-based novel proteins, cultured meat, seaweed or macroalgae, and insects are examples of substitute proteins. In recent times, SCP technology has grown in popularity. As a protein supplement inhuman diets or animal feeds, SCP refers to dead, dried microbial cells or total protein extract from pure microbial cultures of algae, bacteria, filamentous fungus, unicellular algae, and cyanobacteria that are grown on various carbon sources (Reihani *et al.*, 2019).

SCP can be used in place of traditional plant and animal protein sources in the diets of humans, animals, and fish, even though animal proteins are often thought of being high quality proteins.The WHO/FAO-recommended adult essential amino-acid requirement and scoring pattern have been met by SCP or microbial protein. SCP includes larger amounts of important amino acids, including lysine and methionine, which are limiting in many plant and animal proteins, in addition to high levels of protein (60-82% of dry cell weight) and other nutrients.

Industrial wastewater, agricultural waste, petroleum residues such as fuel oil and n-paraffins, methane, heptane, methanol, biogas, CO_2, ethanol, methane, molasses, brewery residue, cellulosic biomass, and many other industrial and agricultural residues are potential feedstockfor SCP production (Jones *et al*., 2020). Due to its greater protein content and shorter microorganism growth cycles, which result in rapid biomass production, microbial protein grown on agricultural wastes is becoming one of the most important protein supplements. Additionally, microbes can develop on inexpensive nutritional sources, producing economically advantageous protein supplements for a balanced diet.

Substrate for SCP production:

The varieties of substrate and the makeup of the culture medium, which have a significant impact on cell growth rate, determine the degree of SCP formation (Hezarjaribi *et al.,* 2016). Materials utilized to produce SCPs should be harmless, plentiful, regenerable, nonexotic, and affordable include nutrients and carbohydrates and are able to enable rapid growth.

The substrates have included both conventional materials like starch, molasses, lignocellulosic biomass, fruit and vegetable wastes, and brewery wastes as well as unconventional ones including petroleum by-products, natural gas, ethanol, methane, and methanol. For the creationof SCP (Reihani *et al.,* 2019). Agricultural wastes have received more attention in a variety of applications because they are inexpensive, naturally abundant, nontoxic, and renewable resources. Orange peel, coconut waste, grape waste, sugar beet pulp, and straw scraps, mango waste, sweet orange waste, and debris. Whey, sugarcane bagasse, rice husk, wheat bran, and other agricultural and agro-industrial waste materials have all been successfully used in the production of SCP and other metabolites (Mensah *et al.,* 2016).

The energy and carbon sources available to microbes as well as their capacity to use those sources are crucial elements that affect their ability to develop. Microorganisms like

bacteria, fungus, yeast, and algae make use of reasonably priced energy and carbon from source materials like starch, lignocelluloses, and organic waste sources for cell development. Raw materials occasionally need to be pre-treated or hydrolysed prior to use. Few other wastes thatcan be potentially used as a substrate for SCP production are listed:

Spent grain:

Brewery spent grain only serves as food for the most basic organisms. It makes up around 85% of the entire byproduct produced in a brewery and is mostly composed of carbohydrate, fibers, minerals, and a small bit of protein. However, it may be repurposed by growing microbes through solid-state fermentation to boost the substrate's protein content and nutritional value, incorporating value to agro-industrial waste in the course of the procedure (Canedo *et al*., 2016).

Stick-water:

When proactively making fish meal, a significant volume of stick water is created because of the high COD. Therefore, with rising disposal and pollution issues, wastewater treatment is expensive. It is crucial to choose a cheap substrate from an industrial process in order to lower the cost of SCP manufacturing by microorganisms. This issue was solved by using wastewater as a substrate for bacteria to produce valuable chemicals like SCP. (Kam *et al.,* 2012).

Dairy waste water:

More than 13% of the milk produced worldwide is produced in India. Dairy waste can also function as a possible source of SCP. Lactose, nitrogenous elements, minerals, and a negligible quantity of vitamins are present. The main source of pollution damaging the ecology is the waste water discharge from milk processing plants. By creating protein-rich products that will serve as a protein supplement for the growing global population, it is vital to determine its nutritional value and acceptability for use as a substrate for SCP manufacturing.

Vegetable and fruit waste:

One of the factors contributing to environmental contamination is food industry waste, Wheat straw, sugar beet pulp, cheese whey, mango seeds, pomegranate rinds, coconut husks, orange, banana, and papaya peels are a few examples of carbohydrate-rich substrate that are frequently utilized in the synthesis of SCP (Thiviya *et al.,* 2021).

Fruit and vegetable waste substrate has several advantages:

1. Lower transportation and expenditure for disposal

2. Create a full fermentation feedstock that doesn't require additional nutrients.
3. The capacity of low-capacity food production facilities to economically use their waste bydelivering it to centralized facility for the treatment of food waste.
4. Strengthening the nutritional value of animal feed affordable agricultural-waste, hence reducing the environmental impact of dumping organic waste (Theodoros Aggelopoulos *et al.*, 2014).

Cultivation methods for SCP production:

Three methods are commonly used to culture microorganisms for SCP production: solid, semisolid, and submerged fermentation

Solid - state fermentation:

The most popular fermentation method for the synthesis of SCP is known as solid-state fermentation. Microbes are grown on solid surfaces with very little moisture to produce fermentation. The insoluble substrate offers vital nutrients including carbon, nitrogen, and others. The substrate that is inexpensive and easily accessible for this fermentation method includes rice husk, wheat bran, sugar beet pulp, wheat and maize flour, etc. Although filamentous fungi are the best microorganisms for solid state fermentation, other microbes such as algae, bacteria, fungi, and yeast can also thrive on solid substrate. This fermentation procedure is the best option for fermentation processing since it is done in batches; if a batch is contaminated, only the tray is lost (Theodoros Aggelopoulos *et al.,* 2014). This method hasmore edge compared to other technique such as:

1. Substrate needs less pretreatment.
2. The medium is simple, readily accessible and reasonably priced
3. Because of low moisture content, contamination is reduced.
4. Straightforward downstream processing that uses the least amount of water possible.
5. Great productivity in terms of volume.
6. Sterile air usually circulates during aeration, not agitation.
7. Substrate are employed gradually and steadily, allowing them to be used for longerperiod of time.

Submerged fermentation:

Microorganisms are grown during submerged fermentation in liquid media that contains more than 95% water. Appropriate fermentation method employing stick water

waste and dairy wastewater to produce SCP. A sizable aseptic vessel that offers a regulated atmosphere, the ideal ph, a certain level of agitation, and the right oxygen content. High water content and uniform distribution of all nutrients make the liquid media for submerged fermentation ideal for microorganisms. Fermentation can be done continuously or in batches. The system may be aerated and stirred using an impeller and sparger (Bakratsas *et al.,* 2023). The following is a list of the benefits of this fermentation process:

1. Easiness of measuring process parameter.
2. Even distribution of nutrients and microorganisms.
3. Ability to control growth conditions.
4. Access to high water content that is conducive to microbial growth.

Additionally, fermenters are divided into three categories according to how they operate: batch fermentation, fed-batch fermentation, and continuous fermentation. In the batch fermenter, broth is removed at the conclusion of the process after the microbial culture has been inoculated to a specific volume of media, and feeding rates regulate the delivery of nutrients in the fed- batch fermenter. Continuous fermentation, in which new medium is continuously provided andboth the old medium and cells are harvested at the same time is ideal for the creation of biomass. Aerators are included in fermenters to provide oxygen for aerobic processes, stirrers to mix the medium, thermostats to manage temperature, ph detectors, and other control devices to maintain the many parameters necessary for consistent growth.

After fermentation, the biomass is cleaned, dried, and either combined with animal feed or usedright away. Typically, only 1-5% of fermentation products are solid. Therefore, pre- concentration is necessary to speed up the dehydration process. Centrifugation followed by heating, filtration, and evaporation are just a few methods for pre-concentration. To enable handling and reduce shipping costs, the finished product should be in a dry powder state. The least expensive ways for removing water are drum drying and spray drying, based upon the expenditure. The finished product should be suitable for human consumption by being light incolour, highly soluble, nutritious, and devoid of viable cells. Additionally, the breakdown of the cell wall and the decrease of the nucleic acids would improve the digestibility and flavorthe dried biomass is encapsulated in a vacuum or nitrogen atmosphere, depending on the manufacturer and the type of product.

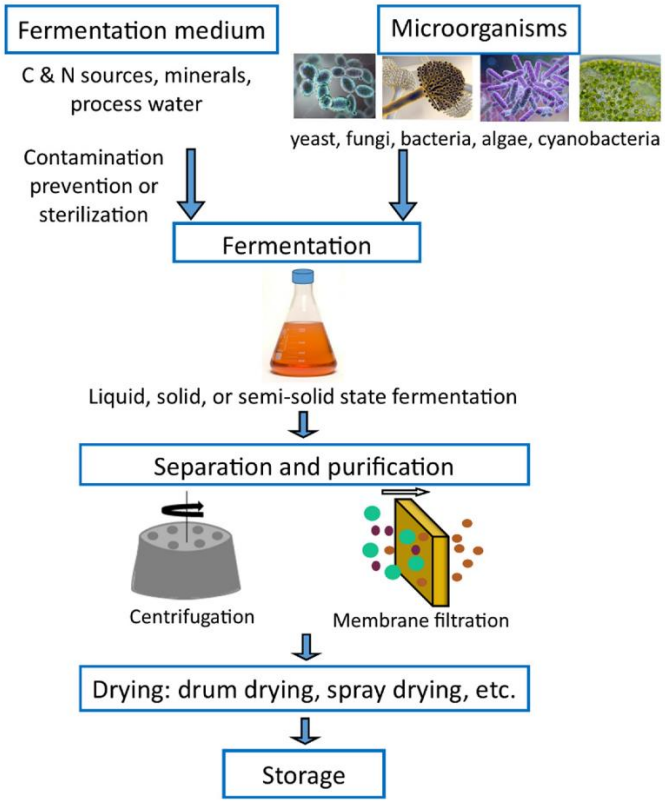

Figure 1: Schematic diagram depicting the SCP production

Factors affecting the SCP production:

Multiple variables, which includes microorganism type, inoculum age, culture media composition, including carbon and nitrogen sources, substrate concentration, incubation temperature, medium ph, the moisture content of solid cultures, dissolved oxygen, and aerationrate, are expected to have an effect on the yield and productivity of SCP production (Mensah *et al.,* 2016).

For the development of scps, microbial strains with a short generation time, high nutritional value, low nucleic acid content, good digestibility, and tolerance to ph are always taken into consideration. Additionally, they should be able to thrive on various complicated substrates and not produce any pathogens or toxic substances, or be designated as GRAS (generally recognized as safe). In addition, tolerance to high cell density, simplicity of handling, stability in growth rate during continuous cultivation, and good organoleptic features are qualities that make microorganisms suitable for SCP manufacturing.

Numerous research examined the impact of process variables, such as pH, temperature, substrate concentration, and fermentation time, on the formation of SCP

employing different microorganism (Kamal *et al.,* 2019). When compared to bacteria, fungus often require a lower ph for growth (Sharif *et al.,* 2021).

Challenges of using SCP:

The majority of SCP products are used as animal feed and are not intended for human consumption; hence, they must adhere to safety standards. It takes even longer and costs moreto gain regulatory approval to produce proteins for human use, which obviously affects the choice of production organism. Nucleic acid content is a safety factor that must be taken into account for all SCP products. Many microorganisms have naturally high nucleic acid levels, but because fermentation conditions that favour rapid growth rates and high protein content also promote elevated RNA levels can be problematic because the digestion of nucleic acids by people and animals results in the production of purine compounds, this can be a problem. Increased plasma levels of uric acid from their subsequent metabolism can cause kidney stonesor gout-like symptoms in the joints. It is also important to look for signs of sensitivity or allergic reactions to the microbial protein as well as signs of sluggish digestion or indigestion of particular microbial cells in the gut. Another issue is the ingestion of poisonous or hazardoussubstances, like polycyclic (Thiviya *et al.,* 2021).

Conclusion:

SCP show incredibly appealing qualities as a nutrient supplement for both animals and plants. They are independent of seasonal and climatic change, allowing for year-round production. SCP can be made from a variety of cost-free substrates. To manufacture SCP, strains with high yield and good composition might be chosen. Less land area is needed, and the environment benefits. This leads to the conclusion that it can simply replace traditional animal and plant protein in the diet of both humans and animals. There are numerous additional uses for single cell proteins. It is utilized as a nutritious snack to prevent obesity and to provide people—especially athletes—immediate energy. Additionally, it is utilized in technical fields as a foam stabilizer and for the processing of paper and leather (Mensah *et al.,* 2016).

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SMALL RNAs: A NEW PARADIGM IN PLANT MICROBE INTERACTIONS

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Introduction:

Plants have evolved multiple layers of immune responses to detect and defend themselves against various invading pathogens such as bacteria, fungi, oomycetes, and viruses. The first layer involves the detection of conserved pathogen-associated molecular patterns (PAMPs), which triggers PAMP-triggered immunity (PTI) that limits pathogen spread. In order to survive and propagate, some pathogens have evolved effector proteins, which can suppress PTI. To prevent infection, plants have evolved resistance (R) proteins that recognize the effectors and activate effector-triggered immunity (ETI), a more rapid and robust immune defense response (Chisholm *et al.,* 2006; Jones and Dangl, 2006). Increasing evidence has shown the important roles of small RNAs (sRNAs) in the regulation of these intricate defense responses against pathogens (Agarwal and Jin, 2010)

sRNAs are short, noncoding RNA molecules that guide silencing of genes either through transcriptional gene silencing (TGS) or post-transcriptional gene silencing (PTGS) (Baulcombe, 2004). They are divided into two subgroups, micro-RNAs (miRNAs) and short interfering RNAs (siRNAs), based on their origin and biogenesis. In plants, siRNAs can be further categorized as trans-acting siRNAs (ta-siRNAs), heterochromatic siRNAs (hc-siRNAs), natural antisense transcript-derived siRNAs (nat-siRNAs), or long siRNAs (lsiRNAs) (Chapman and Carrington, 2007).

sRNA discovery

The founding member of the miRNA family, lin-4, was identified in *C. elegans* through a genetic screen for defects in the temporal control of post-embryonic development. In *C.elegans*, cell lineages have distinct characteristics during 4 different larval stages (L1–L4). Mutations in lin-4 disrupt the temporal regulation of larval development, causing L1 (the first larval stage) specific cell-division patterns to reiterate at

later developmental stages (Ambros, 1989). Small RNAs are 20 to 40 nucleotide (nt)-long noncoding RNA molecules present in most eukaryotic organisms that regulate gene expression in a sequence-specific manner either transcriptionally or post transcriptionally. On the basis of their biogenesis and precursor structure, small RNAs are placed in two distinct groups viz. microRNAs (miRNAs) and small interfering RNAs (siRNAs). Small RNAs regulate a multitude of biological processes in plants, including development, metabolism, maintenance of genome integrity, immunity against pathogens, and abiotic stress responses. Increasing evidence suggests that small RNAs play a critical role in regulating the interaction of pathogens with plants (Baulcombe, 2004).

Small RNAs are the primary actors in RNA silencing. They selectively guide the RNA-induced silencing complex (RISC) to specific transcripts resulting in degradation of numerous genes involved in physiological and developmental processes, defense against selfish repetitive elements and invading parasites (Malone and Hannon, 2009)

Core components of small RNA biogenesis pathway

1. DCL and dsRNA-binding proteins

Dicer Like proteins (DCL) belong to the RNase III family of endoribonucleases that specifically process dsRNAs into sRNA duplexes. DCL typically contain a helicase domain, a DUF 283 domain, a PAZ domain, two tandem RNase III domains, and two tandem dsRNA-binding domains (Carmell and Hannon 2004). Arabidopsis encodes four DCL (DCL1 to DCL4. DCL1 predominantly generates miRNAs but is also involved in producing some endogenous siRNAs, such as natsiRNAs and lsiRNAs (Zhang *et al.,* 2012). DCL2, DCL3, and DCL4 mainly process long dsRNA precursors produced by RDR, natural antisense transcription, or inverted repeats. DCL3 is responsible mainly for the processing of Pol IV- and RDR2-generated dsRNAs and gives rise to hc-siRNAs that guide RdDM at target genomic loci. DCL4 takes part in the generation of predominantly 21-nucleotide (nt) ta-siRNAs in an RDR6-dependent manner (Chapman and Carrington 2007). A recent study revealed that DCL4 also has a siRNA-independent function in cleaving nascent transcripts and promoting transcription termination of the FCA gene (Liu *et al.,* 2012).

In antiviral immunity, all four DCL are involved in producing virus-derived siRNAs (viRNAs). DCL4 has the predominant role in antiviral defense and targets dsRNAs derived from virus replication to produce 21-nt viRNAs, whereas DCL2 acts as an alternative enzyme in a hierarchical manner to produce 22-nt viRNAs. DCL4 is sufficient for antiviral silencing in inoculated leaves but both DCL2 and DCL4 are involved in antiviral silencing in systemic tissues. DCL3 produces 24-nt viRNAs in plants infected with DNA viruses.

Although the role of these 24-nt viRNAs in antiviral resistance is controversial, recent studies have shown that geminiviruses encode suppressors that specifically target the DNA methylation process.

2. RNA-dependent RNA polymerases

In plants, RDRs are important for siRNA formation because they synthesize dsRNAs for downstream processing by DCL. Initial studies showed that virus infection enhances RDR activity, which led to the hypothesis that RDRs are one of the many host factors that assist virus replication. Subsequently, extensive studies have implicated RDRs in antiviral defense in plants. RDR1 activity is induced not only by virus infection but also by defense signalling compounds such as salicylic acid (SA). Reducing the expression levels of RDR1 in transgenic antisense Arabidopsis plants resulted in enhanced accumulation of viral RNAs and increased susceptibility to TMV and potato virus X(PVX) infection. AtRDR1,an ortholog of NtRDR1, is also known to impart defense against tobamovirus and tobravirus because Arabidopsis rdr1 mutant plants had enhanced levels of viral RNAs (Yu *et al.,* 2003). NtRDR1 is also involved in combating potato virus Y(PVY) infection; knocking down expression of RDR1 in transgenic tobacco plants resulted in reduced expression of other defense-related genes such as AOX1 and ERF5.

Small RNA profiling revealed that RDR1 preferentially amplifies viRNAs that mapped at the 5'-terminal viral RNAs, whereas RDR6-dependent viRNAs mapped to the 3'-terminal half of viral RNAs. RDR6 interacts with a coiled-coil protein, SGS3, to produce secondary viRNAs (Kumakura *et al.,* 2009). An sgs3 mutant shows enhanced susceptibility to cucumovirus, which indicates that SGS3 also contributes to antiviral resistance in plants. The generation of nat-siRNAATGB2 and AtlsiRNA-1 requires RDR6. Pst DC3000 (avrRpt2) displayed enhanced growth in the rdr6 mutant, which provided direct evidence for the function of RDR6 in plant immunity.

3. Argonautes

AGOs are associated with small RNAs and form RISC complexes to induce silencing of target genes (Hannon, 2002). Arabidopsis contains 10 AGOs, and their role in plant immunity is yet to be determined. There is emerging evidence that the methylation status of plant genomes is altered in response to attack by pathogens, including viruses, bacteria, and fungi (Pavet *et al.,* 2006). hc-siRNAs trigger transcriptional gene silencing (TGS) by guiding RNA-directed DNA methylation (RdDM) and histone modification in plants (Matzke and Birchler, 2005). AGO4 is a major nuclear RNAi effector associated with hc-siRNAs or rasiRNAs that direct DNA methylation (Qi *et al.,* 2006). Involvement of AGO4 in the disease-

resistance response links DNA methylation and plant defense. When attacked by viruses, plants employ DNA methylation to repress viral transcription and/or replication. Upon infection by either of two geminiviruses, cabbage leaf curl virus (CaLCuV) or beet curly top virus (BCTV), Arabidopsis plants silence viral chromatin by both cytosine and histone methyl transferases. This is evident from the hypersusceptibility of the methylation-deficient mutants to geminiviruses, including mutants of cytosine methyl transferases (drm1, drm2, cmt3, and met1), histone H3K9 methyl transferase (kyp2), RdDM pathway components (ago4, ddm1, and nrpd2A), or methyl cycle enzymes (adk1 and adk2). Viral suppressors AL2 and L2 inhibit the activity of adenosine kinase (ADK), a cellular enzyme involved in the generation of S-adenosyl methionine (a methyl transferase cofactor). Therefore, plants infected with virus lacking L2 had hypermethylation of viral DNA. Additionally, recovery of the virusinfected plants from the disease symptoms required AGO4 (Raja *et al.,* 2008).

Biogenesis pathway of small RNAs

1. miRNA pathway

miRNAs are derived from the transcripts of miRNA genes generated by RNA polymerase II. The primary miRNA (pri-miRNA) transcript forms a fold-back structure, which is processed into a stem-loop precursor known as precursor miRNA (pre-miRNA). A protein named DAWDLE (DDL) has been proposed to play an important role in miRNA biogenesis by recruiting predominantly DICER-like protein 1 (DCL1) to pri-miRNA for downstream processing. The pre-miRNA is acted upon by DCL1 together with HYL1 (HYPONASTIC LEAVES 1) and SE (SERRATE) to form the small RNA duplex. The small RNA duplex is then methylated at the 3' ends by HEN1 (HUA ENHANCER 1) and is exported to the cytoplasm by an exportin homolog, HST (HASTY). Mature miRNA is preferentially incorporated into AGO1 (or AGO10) and guides the complex to the target mRNA for cleavage or translational inhibition on the basis of sequence complementarity.

2. siRNA pathways

In contrast to miRNAs that are derived from imperfectly base-paired hairpin loop structures, siRNAs are derived from perfectly paired double-stranded RNA (dsRNA) precursors. These dsRNA precursors are derived either from antisense transcription or by the action of a cellular RNA-dependent RNA polymerase (RDR). Four different types of siRNAs are known in plants: trans-acting siRNAs (ta-siRNAs), natural antisense transcripts (NATs)-derived siRNAs (nat-siRNAs), heterochromatic siRNAs (hc-siRNAs) or repeat-associated siRNAs (ra-siRNAs), and long siRNAs (lsiRNAs). RNA Pol II transcribes

noncoding TAS genes, and the long primary transcript products are initially cleaved by miRNAs loaded with RNA-induced silencing complexes (RISCs), resulting in a 5' fragment or a 3' fragment. These fragments then act as a template for synthesis of a complementary strand by the concerted action of RDR6 and SGS3. The resulting dsRNA molecule is acted upon by DCL4 and DRB4 to trigger the subsequent production of ta-siRNAs. nat-siRNAs are produced from the overlap regions of sense and antisense transcripts of cis-NATs. A significant proportion of most eukaryotic genomes encode overlapping cis-NAT genes, which have the potential to generate siRNAs when base pairing between sense and antisense transcripts occurs. Though natsiRNAs have been shown to play an important function in both abiotic and biotic stresses, their roles in other plant processes remains to be investigated. The cellular components involved in production of nat-siRNAs are DCL1 and/or DCL2, HYL1, and HEN1. The nat-siRNAs studied also partially depend on RDR6, SGS3, and Pol IV. hc-siRNAs or ra-siRNAs are usually 24 nt in length and are primarily derived from transposons, repeat elements, and heterochromatin regions. Their biogenesis is dependent on the DCL3-RDR2 Pol IV pathway. hc-siRNAs or ra-siRNAs function in the RNA-dependent DNA methylation (RdDM) pathway by mediating DNA methylation and/or histone modification at the target sites. In addition to 21 to 24 nt siRNAs, a class of lsiRNAs in the size range of 30 to 40 nt was discovered. The biogenesis of lsiRNAs is dependent on DCL1, HYL1, HEN1, AGO7, and HST and partially dependent on RDR6 and Pol IV. AtlsiRNA-1 is induced by bacterial pathogen Pseudomonas syringae and triggers silencing of the target by destabilizing the target mRNA through decapping and 5'-3' degradation.

3. Host sRNAs

Many plants endogenous sRNAs, including microRNAs (miRNAs) and small interfering RNAs (siRNAs), play an important role in gene expression reprogramming and fine-tuning in host immune responses (Jin, 2008). Most miRNAs are 20–22 nts in length and are processed by DCL proteins from a single-stranded RNA (ssRNA) precursor with a hairpin structure. The model plant Arabidopsis genome encodes four DCL sand ten AGOs. Most Arabidopsis miRNAs are generated by DCL1 and loaded into AGO1 to induce mainly posttranscriptional gene silencing (PTGS). Plant endogenous siRNAs are numerous in quantity and are much more diverse than miRNAs in terms of their length and biogenesis pathways. siRNAs are processed from long dsRNA precursors by different DCLs and are loaded into different AGOs to guide silencing of their targets transcriptionally or post transcriptionally. Trans-acting siRNAs (tasiRNAs), natural antisense transcript-derived

siRNAs (nat-siRNAs), long siRNAs (lsiRNAs), and heterochromatic siRNAs (hcsiRNAs) are different siRNA subclasses present in plants (Axtell, 2013).

Plant sRNAs are differentially expressed during pathogen attacks. Arabidopsis miR393 was the first miRNA discovered to be involved in plant immunity. Some conserved miRNAs, such as miR160 and miR167, are also induced by PAMPs and contribute to PTI. However, other miRNAs, such as miR398a and miR773, are downregulated upon PAMP perception and bacterial infection to release target suppression and activate PTI. Plant sRNAs also play an important role in ETI. Arabidopsis nat-siRNAATGB2 was the first example of a plant endogenous siRNA that regulates R-gene mediated immunity. It is highly induced by the bacterial pathogen *Pseudomonas syringae pv. tomato* (Pst), which carries an effector gene, avrRpt2, and contributes to resistance gene RPS2-mediated ETI by repressing a putative negative regulator of the RPS2 pathway.

Plant endogenous sRNAs that are responsive to fungal infection have also been identified from many plant species. In rice (Oryza sativa), sRNA profiling of the rice blast fungus *Magnaporthe oryzae*–challenged resistant and susceptible cultivars revealed a group of host endogenous miRNAs that contributes to gene regulation of defense responses.

NBS-LRR gene family sRNAs

Most plant R genes belong to the nucleotide-binding site (NBS) leucine-rich repeat (LRR) gene family, which is a hot spot for sRNA generation. A class of phased secondary siRNAs has been identified from the NBS-LRR type of R-gene regions in various plant species (Shivaprasad *et al.,* 2012). The biogenesis of these secondary siRNAs is initiated by cleavage of the NBS-LRR transcripts mediated by specific miRNA families, such as miR482 and miR2118 from both tomato (*Solanum lycopersicum*) and cotton (*Gossypium hirsutum*) and miR6019 and miR6020 from tobacco (*Nicotiana benthamiana*). These miRNAs and secondary siRNAs control the expression of NBS-LRR genes at a low level under normal conditions. However, this silencing effect is suppressed when plants are under attack from viral and bacterial pathogens, which leads to the upregulation of these R genes and triggers plant defense responses.

sRNA regulation

Certain plant endogenous small RNAs (miRNAs and siRNAs) are up or down-regulated by pathogen infection and target R genes or defense responses genes in plant immunity. These small RNAs contribute to gene expression reprogramming and fine-tuning in plant resistance and defense responses (Jin, 2008).

Pathogen derived sRNAs

Bacterial sRNAs are completely different from the sRNAs found in eukaryotes because of the fact that prokaryotes do not possess DCLproteins. Instead, bacterial regulatory noncoding sRNAs are heterogeneous in length (50–300nts) and regulate the stability and translation efficiency of target mRNAs through short and imperfect base-pairing(10–25nts). They are often functionally associated with RNA-binding protein complexes. Although several high-throughput RNA-sequencing studies have identified potential sRNAs in phytopathogenic bacteria, including *Agrobacterium tumefaciens*, *Pst*, *Xanthomonas campestris* and *Xanthomonas oryzae pv. oryzae*, defined functions in pathogenesis remain scarce. Recently, an elegant study using genome-wide transcriptome analysis revealed that *X. campestris pv. vesicatoria*, the causal agent of bacterial spot disease in pepper (*Capsicum annuum*) and tomato, produces noncoding sRNAs. Some are under the control of HrpG and HrpX, two regulatory proteins of the type III secretion system, which is essential for bacterial pathogenesis (Schmidtke *et al.,* 2012). Gene deletion analysis demonstrated that the sRNAs sX12 and sX13 contribute to virulence. sX13 promotes the synthesis of HrpX and regulates expression of other proteins putatively involved in signal transduction, motility, transcriptional and posttranscriptional regulation, and virulence. Bacterial noncoding sRNAs operate with RNA-binding protein complexes, the most prominent of which are the clustered regularly interspaced short palindromic repeat (CRISPR) CRISPR-associated (Cas) system (CRISPR-Cas), the global regulatory protein Hfq, and the CsrA/RsmA RNA-binding protein.

Hfq is a hexameric RNA-binding protein that acts as a global posttranscriptional regulator by binding to bacterial sRNAs to inhibit translation or promote degradation of target mRNAs (Vogel and Luisi, 2011). Hfq protein is present in approximately 50% of all bacteria and is found in most plant pathogenic bacteria, including *A. tumefaciens, Ralstonia solanacearum, Pectobacterium carotovorum, P. syringae, and Xanthomonas spp.*

The CsrA/RsmA protein is a dimeric prokaryote-specific regulatory sRNA-binding protein that affects translation or the stability of target mRNAs (Heroven *et al.,* 2012). CsrA/RsmA protein genes are found in many pathogenic bacteria, including plant pathogens such as *X. campestris and P. carotovorum*.

Localization of effector genes

Genomic regions rich in TEs are normally poor in protein-coding genes, but TE-rich regions from these phyto pathogens show a remarkable enrichment of virulence genes, such as protein effector genes (Sacristan *et al.,* 2009). Consistent with this notion, *B. cinerea*

sRNA effectors are also derived from LTR-transposon regions. Although massive expansion of transposons has not occurred appreciably in the *B. cinerea* genome, population dynamics surveys and genetic marker analysis of *B. cinerea* species linked the presence of two TEs, the LTR retrotransposon Boty and Fot1-like element FLIPPER, to the virulent and host-adapted subpopulations named transposa isolates. Similarly, rice isolates of *M. oryzae* collected from geographically dispersed locations exhibit high copy numbers of the LTR retrotransposon MAGGY compared with non-rice isolates collected from other Gramineae. TEs are also likely to shape plant R-gene evolution. Many R genes, in distinct plant species, are clustered within certain genomic loci and are often close to TEs and repeats, thus facilitating DNA mutation upon selection pressure. The TEs carry epigenetic information to control the expression of these R genes, as demonstrated for the Arabidopsis RPP5 and RPP7R-gene loci (Tsuchiya and Eulgem, 2013). R-gene clusters are also hot spots for sRNA generation. sRNAs play an essential role in regulating the epigenetic status of R-gene loci and fine-tuning the expression of R genes during infection. TEs and sRNAs are indispensable regulators in the arms race between eukaryotic pathogens and hosts.

Cross-kingdom RNAI

A form of RNAi in which a gene-silencing trigger is produced in a donor organism but mediates gene silencing in an unrelated recipient organism. Fungal sRNA effectors are delivered into host cells to suppress host immunity genes and achieve successful infection. This silencing event represents a naturally occurring cross-kingdom RNAi event, which was discovered in a fungal pathogen as an advanced virulence mechanism. sRNAs moving in the opposite direction from the plant host cells to the fungal pathogen, plant-parasitic organisms, or insect pest cells have also been observed. Silencing signals produced from transgenic plants expressing RNAi constructs that target genes of nematodes, insects, parasitic plants, and fungal pathogens can translocate into and induce gene silencing within the pest, parasite, or fungal cells. This so-called host-induced gene silencing (HIGS) has become a useful tool to study gene function of non-transformable pests and pathogens and to develop resistance in crops (Nunes *et al.,* 2011; Price and Gatehouse, 2008). HIGS has provided an excellent example of cross-kingdom RNAi by transferring silencing signals from host to pathogens/pests. Recently, miRNAs from plants consumed as food were detected in human and animal sera, suggesting that cross-kingdom RNAi can occur in various systems.

Conclusion:

1. Small noncoding RNAs and RNAi pathways play a crucial role in regulating plant pathogen interactions, including plant immunity and pathogen virulence.
2. sRNAs act as effector molecules to suppress host immunity by hijacking host RNAi pathways.
3. To confer an evolutionary advantage, pathogen effector genes, including sRNA effectors, and plant host R genes often colocalize with TEs. TEs are hot spots of sRNA production.

Different components of small RNA pathways directly play important roles in mediating host immune responses against pathogens.

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AB-QTL ANALYSIS: A MAJOR APPROACH OF MOLECULAR PLANT BREEDING

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The advanced backcross (AB) QTL method, which integrates QTL analysis with variety development to simultaneously identify and develop a domestic target population for mapping a desirable trait found in the wild/exotic parent and transfer the trait into the cultivated parent, was suggested by Tanksley and Nelson in 1996 (Tanksley and Nelson, 1996). Molecular plant breeding is a specialized field within plant breeding that integrates molecular biology techniques and genomics to enhance the efficiency and precision of crop improvement. It leverages genetic markers, DNA sequencing, and other molecular tools to expedite the breeding process and develop new plant varieties with improved traits. Modern plant breeding faces the complex challenge of developing crop varieties that not only meet the increasing global demand for food, fiber, and bioenergy but also adapt to changing environmental conditions, resist emerging pests and diseases, and exhibit improved nutritional profiles. To achieve these goals efficiently and effectively, plant breeders have turned to advanced techniques, and one such technique that has gained prominence is Advanced Backcross QTL (AB-QTL) analysis. AB-QTL analysis represents a powerful and indispensable tool in the arsenal of modern plant breeding. It combines the principles of traditional backcrossing with molecular and genomic technologies to expedite the development of crop varieties with targeted traits. This approach has revolutionized the field by enhancing precision, accelerating breeding cycles, and improving the overall efficiency of crop improvement programs.

Here's a brief overview of molecular plant breeding:

a) **Genetic marker development**: Molecular plant breeding relies on the development and use of genetic markers, which are specific DNA sequences associated with particular traits. Common markers include SNPs, SSRs, and InDels. These markers are used to identify and track genes or QTLs (Quantitative Trait Loci) linked to desirable traits in plants.

b) **Traits mapping and QTL analysis:** Molecular techniques are employed to map the

locations of genes and QTLs responsible for specific traits within a plant's genome. QTL analysis helps breeders pinpoint the genomic regions associated with traits they want to improve, providing critical information for breeding programs.

c) **Marker Assisted Selection (MAS):** MAS involves the use of genetic markers to identify plants with desired traits at an early stage, such as during seedling development. This speeds up the breeding process by allowing breeders to select individuals more likely to possess the desired traits, reducing the need for extensive field trials.

d) **Genomic selection:** Genomic selection utilizes information from the entire genome to predict a plant's potential performance. It is particularly valuable for complex traits influenced by multiple genes, as it considers the collective genomic data rather than individual markers.

e) **Genomic resources:** The availability of plant genome sequences and extensive genomic databases has transformed molecular plant breeding. These resources provide detailed genetic information about target crops, facilitating trait discovery, and marker development.

f) **Targeted traits:** Molecular plant breeding can be applied to a wide range of traits, including disease resistance, drought tolerance, nutritional content, and overall yield improvement. The specific traits of interest depend on the crop and breeding objectives.

g) **Speed and precision:** Molecular plant breeding accelerates the breeding process, reducing the time required to develop new crop varieties. It also allows breeders to make more informed decisions, leading to greater precision in selecting plants for further breeding.

h) **Ethical and regulatory consideration:** Molecular breeding may involve genetic modification (GM) techniques in some cases, which raises ethical and regulatory questions about the use of GM crops.

Significance of QTL analysis in crop improvement.

Quantitative Trait Locus (QTL) analysis is of immense significance in crop improvement for several key reasons:

a) **Identification of genes and traits:** QTL analysis helps researchers and breeders pinpoint the specific genomic regions associated with important traits in crops. These traits can include yield, disease resistance, tolerance to environmental

stresses (such as drought or heat), and nutritional content. Identifying the underlying genes is the first step in understanding the genetic basis of these traits.

b) **Precision in breeding:** QTL analysis provides precise information about the location and effect of genetic factors influencing a trait. This precision enables breeders to make informed decisions when selecting parent plants for crosses, resulting in more efficient and targeted breeding programs.

c) **Accelerating breeding cycles:** Traditional crop breeding can be time-consuming, requiring multiple generations and extensive field trials. QTL analysis, particularly when combined with marker-assisted selection (MAS), allows for the early identification of individuals carrying desirable alleles for target traits. This accelerates the breeding process by reducing the need for lengthy field evaluations.

d) **Enhanced trait selection:** Breeders can use QTL analysis to select plants with the highest probability of transmitting desirable traits to their offspring. This increases the likelihood of developing crop varieties with improved characteristics, such as higher yield or better nutritional profiles.

e) **Understanding trait complexities:** Many agronomically important traits, like yield or disease resistance, are controlled by multiple genes and influenced by environmental factors. QTL analysis helps dissect the genetic complexities behind these traits, providing valuable insights into their inheritance patterns and interactions.

f) **Incorporating genetic diversity:** Crop improvement often involves introducing genetic diversity into breeding programs to enhance traits or adapt crops to changing environments. QTL analysis aids in identifying and utilizing genetic variation from diverse germplasm, allowing for the development of more resilient and adaptable varieties.

g) **Targeted genetic modification:** In some cases, QTL analysis can guide genetic modification efforts by identifying specific genes or pathways responsible for desired traits. This knowledge can inform the development of genetically modified crops with improved characteristics.

h) **Sustainable agriculture:** QTL analysis can contribute to sustainable agriculture by enabling the development of crops with reduced input requirements (e.g., less water or fertilizer) and increased resistance to pests and diseases. This can lead to more resource-efficient and environmentally friendly farming practices.

i) **Meeting global challenges:** As the world faces challenges like climate change, population growth, and food security, QTL analysis becomes even more critical. It provides a powerful tool for developing crops that can thrive under changing environmental conditions and meet the increasing demand for food, fiber, and bioenergy.

Principles of QTL analysis

A. The genetic basis of complex traits.

The genetic basis of complex traits refers to the underlying genetic factors and mechanisms that contribute to the inheritance and expression of traits that are multifactorial in nature. Complex traits are characterized by continuous variation within a population and are influenced by the interplay of multiple genes, environmental factors, and gene-environment interactions (Grandillo and Tanksley, 2005). Understanding the genetic basis of complex traits is a challenging but essential aspect of genetics and biology. Here are key components of the genetic basis of complex traits:

a) **Polygenic inheritance:** Complex traits are typically polygenic, meaning they are influenced by multiple genes located on different chromosomes. Each gene may have a small additive effect on the trait, and the combined effects of these genes contribute to the overall variation observed in the trait.

b) **Quantitative Trait Loci (QTL)**: Specific genomic regions associated with the inheritance of complex traits are called Quantitative Trait Loci (QTL). QTL can be identified through techniques like QTL mapping, which involves studying the genetic makeup of individuals with varying trait values and identifying regions of the genome that correlate with the trait.

c) **Allelic variation:** The alleles (different versions of a gene) at each QTL can have different effects on the trait. Some alleles may increase the trait's value (positive effect), while others may decrease it (negative effect). The combination of alleles inherited from both parents determines the trait's final expression.

d) **Gene interaction:** Genes influencing complex traits can interact with each other in various ways. This includes epistasis, where the effect of one gene depends on the presence or absence of alleles at other genes. Gene interactions can amplify, dampen, or modify the overall effect on the trait.

e) **Environmental influence:** Complex traits are also influenced by environmental factors such as nutrition, temperature, and stress. Environmental conditions can

interact with genetic factors, affecting the trait's expression. For example, a plant's height may be influenced by both its genetic makeup and the amount of water it receives.

f) **Gene environment interaction:** The impact of genes on complex traits can vary depending on the environment. This is known as gene-environment interaction. For instance, certain genetic variants may confer resistance to a disease but only under specific environmental conditions.

g) **Phenotypic plasticity:** Some complex traits exhibit phenotypic plasticity, which means that the same genotype can produce different trait values in response to varying environmental conditions. Phenotypic plasticity allows organisms to adapt to changing environments.

h) **Quantitative genetics**: The field of quantitative genetics studies the inheritance of complex traits. It uses statistical models and genetic data to estimate heritability (the proportion of trait variation attributable to genetic factors) and predict how traits will respond to selective breeding.

i) **Genome-Wide Association Studies (GWAS)**: GWAS is a powerful tool for identifying genetic variants associated with complex traits in human populations and model organisms. It scans the entire genome to find associations between specific genetic markers (SNPs) and traits.

j) **Biological pathways**: Genes contributing to complex traits are often part of biological pathways and networks. Understanding these pathways can provide insights into the mechanisms underlying the trait and potential targets for intervention or manipulation.

B. Mapping QTLs in plant genomes

Mapping Quantitative Trait Loci (QTLs) in plant genomes is a crucial step in understanding the genetic basis of complex traits and facilitating crop improvement through selective breeding. QTL mapping allows researchers and plant breeders to identify specific genomic regions associated with traits of interest, such as yield, disease resistance, or stress tolerance. Here's an overview of the process of mapping QTLs in plant genomes:

(i) Trait measurement: The first step is to accurately measure the quantitative trait of interest in a population of plants. For example, if you are interested in yield, you would measure the yield of each plant in your population.

(ii) Population creation: You need a genetically diverse population for QTL mapping. This population typically consists of individuals (plants) that display a range of trait values. This genetic diversity allows you to identify the genetic factors contributing to trait variation.

(iii) Genotypic data: Obtain genotypic data for each individual in the population. This involves genotyping using molecular markers (e.g., SNPs, SSRs) or other genetic markers distributed throughout the plant genome. The genotypic data reveal the genetic makeup of each individual at marker loci.

(iv) Phenotypic and genotypic correlation: Analyze the correlation between the trait measurements (phenotypic data) and the genetic markers (genotypic data). Statistically, you can use methods like regression analysis or ANOVA to assess the association between specific markers and trait variation.

(v) QTL mapping: Identify regions of the genome where the presence of specific markers is significantly associated with trait variation. These regions are the QTLs for the trait. The strength of the association is often expressed as LOD (Logarithm of Odds) scores.

(vi) Confidence intervals: QTL mapping provides confidence intervals for the location of QTLs. These intervals represent the approximate genomic region where the causative genes or genetic variants influencing the trait are located.

(vii) Validation: Validate the identified QTLs by conducting additional experiments or using different populations (e.g., bi-parental crosses, association mapping populations). Confirming the presence and effect of QTLs in multiple genetic backgrounds increases their credibility.

(viii) Fine mapping: In some cases, researchers perform fine mapping to narrow down the QTL region to a smaller genomic interval. This involves using a higher density of markers and more refined genetic mapping techniques.

(ix) Candidate gene identification: Once a QTL is mapped to a specific genomic region, researchers can explore the genes within that region. Candidate genes are those that are known to be involved in the trait of interest or have functions that suggest their involvement.

(x) Functional validation: Functional studies, such as gene expression analysis or transgenic experiments, can be conducted to validate the role of candidate genes in influencing the trait. This helps in understanding the underlying molecular mechanisms.

(xi) Marker Assisted Selection (MAS): If validated, QTLs can be used in marker-assisted selection programs to breed plants with the desired traits more efficiently. Breeders select individuals with favorable QTL alleles based on molecular marker information.

(xii) Trait improvement: Over multiple breeding cycles, the selected plants with favorable QTL alleles can be used to develop crop varieties with improved traits, which may include higher yield, disease resistance, or stress tolerance.

AB-QTL analysis workflow:

A. Step-by-step guide to conducting AB-QTL analysis

Conducting Advanced Backcross QTL (AB-QTL) analysis is a complex but powerful process for identifying and utilizing quantitative trait loci (QTLs) in plant breeding. Here's a step-by-step guide to conducting AB-QTL analysis:

Step 1: Define the objective and trait of interest

Before starting AB-QTL analysis, clearly define the objectives of your breeding program and the specific traits you want to improve. Identify the target traits, such as yield, disease resistance, or stress tolerance, that you aim to enhance through AB-QTL analysis.

Step 2: Choose the recurrent parent and donor line

Select the recurrent parent, which is an elite cultivar with most of the desired characteristics but lacking in certain target traits. Choose a donor line that possesses the target traits you want to introduce into the recurrent parent.

Step 3: Develop the mapping population

Cross the recurrent parent with the donor line to create the mapping population. This population typically consists of F1 hybrids between the recurrent parent and donor line.

Step 4: Backcrossing

Backcross the F1 individuals with the recurrent parent over several generations. This process involves selecting F1 plants that exhibit the desired target trait(s) and repeatedly crossing them with the recurrent parent. The goal is to introgress the target QTL(s) from the donor line into the recurrent parent's genetic background.

Step 5: Phenotyping

Phenotype the individuals in the mapping population for the target trait(s) at multiple stages of development or under different environmental conditions. Accurate phenotypic data are essential for QTL analysis.

Step 6: Molecular marker development

Develop genetic markers, such as SNPs or SSRs, distributed throughout the genome. These markers will be used to genotype the individuals in the mapping population. Markers that are polymorphic between the recurrent parent and donor line are particularly valuable.

Step 7: Genotyping

Genotype the mapping population using the developed molecular markers. This step involves DNA extraction, PCR amplification, and marker analysis. Determine the genetic makeup (genotype) of each individual at the marker loci.

Step 8: QTL analysis

Perform QTL analysis to identify genomic regions associated with the target traits. There are several methods for QTL analysis, including interval mapping, composite interval mapping, and multiple QTL mapping. These methods evaluate the statistical associations between marker data and trait values.

Step 9: QTL validation

Validate the identified QTLs using additional populations or environments to confirm their effects on the target traits. Replication helps ensure the reliability of the identified QTLs.

Step 10: Candidate gene identification

Once QTLs are identified and validated, explore the genomic regions containing these QTLs for candidate genes that may underlie the trait of interest. Sequence analysis and functional studies can be conducted to identify specific genes responsible for the trait.

Step 11: Marker Assisted Selection (MAS)

If QTLs are validated and candidate genes are identified, you can implement MAS in your breeding program. Use molecular markers linked to the target QTLs to select individuals with the desired trait characteristics efficiently.

Step 12: Trait improvement

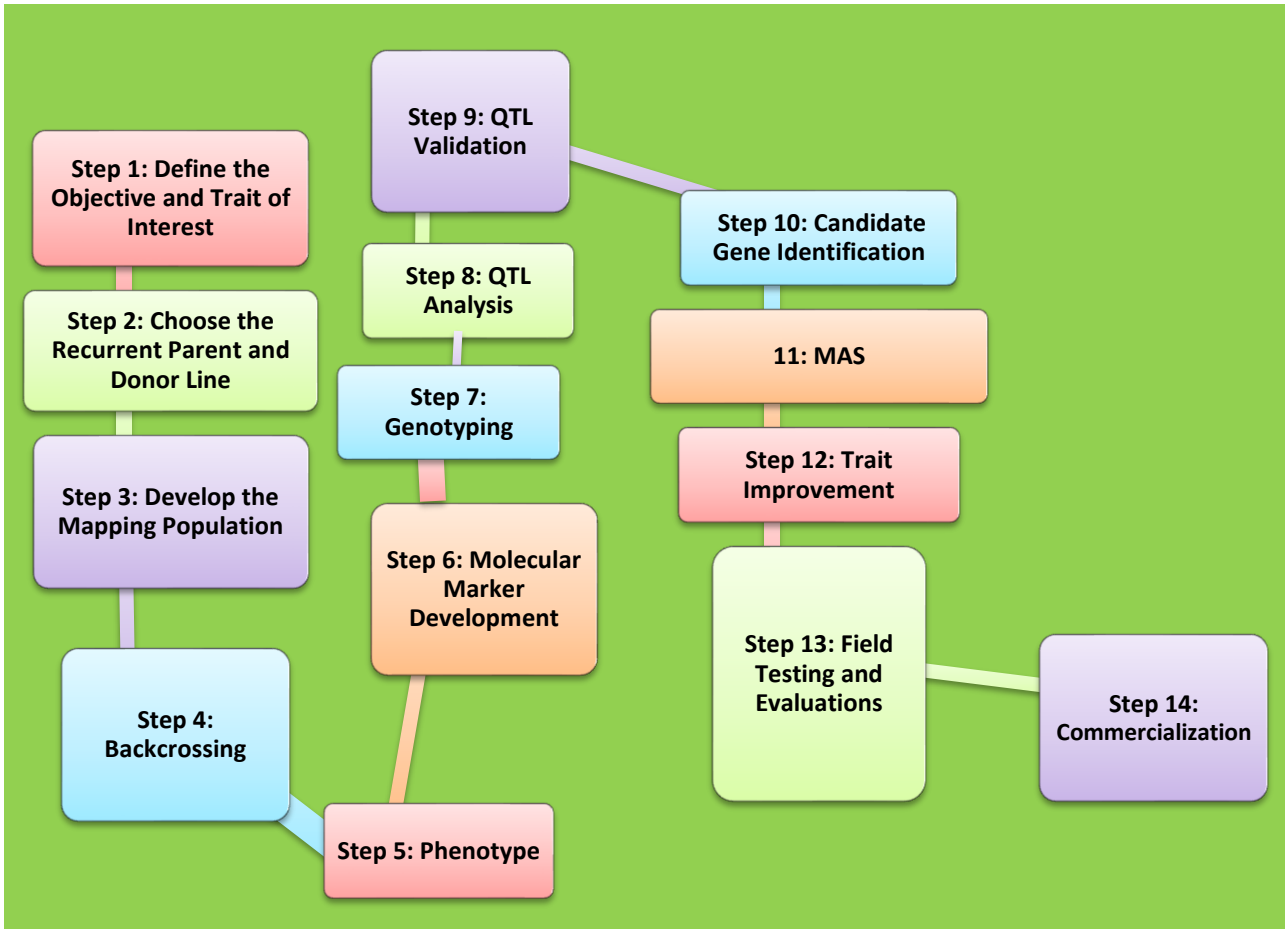
Over several breeding cycles, use the knowledge gained from QTL analysis and MAS to develop crop varieties with improved traits. Continue selecting and breeding individuals with favorable QTL alleles to achieve the desired trait improvements.

Step 13: Field testing and evaluations

Conduct field trials and evaluations of the improved varieties under various environmental conditions to ensure their performance and stability.

Step 14: Commercialization

Once you have developed and tested improved varieties with the desired traits, they can be commercialized and made available to farmers.



B. Selection of parental lines

The first step in any breeding program is selecting the parental lines. These are the plants with the desired traits that you want to combine in the offspring. Parental lines should be genetically diverse and ideally have complementary traits.

C. Genetic marker development and genotyping

Genetic markers are specific sequences of DNA that can be used to track the inheritance of genes from one generation to the next. These markers can be developed through various techniques, such as DNA sequencing, PCR (Polymerase Chain Reaction), or SNP (Single Nucleotide Polymorphism) analysis. Genotyping involves analyzing the genetic makeup of the parental lines and offspring to identify which alleles (gene variants) they carry at specific loci (positions on chromosomes). This information is crucial for understanding the inheritance of traits.

D. Phenotyping and trait evaluation

Phenotyping involves observing and measuring the physical and biochemical characteristics (traits) of the plants, such as yield, disease resistance, drought tolerance, and other desired features. Trait evaluation is a critical step to assess the performance of the offspring. It helps in identifying plants that exhibit the desired traits.

These steps are part of a cyclical process:

(i) **Selection of progeny:** Based on the genetic marker information and phenotypic data, you can select the progeny (offspring) that show the desired traits and have the preferred genetic makeup.

(ii) **Backcrossing and recombination:** In some cases, you might need to perform backcrossing, which involves crossing the selected progeny with one of the parental lines to introduce or enhance a specific trait while retaining most of the desirable characteristics of the other parent. Recombination is the natural shuffling of genes during sexual reproduction, leading to genetic diversity. It is important for creating novel combinations of genes.

(iii) **Repeat the process**: The breeding cycle often requires multiple rounds of selection, genetic marker analysis, phenotyping, and breeding to refine and improve the desired traits.

(iv) **Field testing and validation**: Promising progeny resulting from the breeding process are tested in field trials to ensure that they perform well under real-world conditions, including various environmental factors

(v) **Release and commercialization**: Once a new plant variety has been thoroughly tested and meets the desired criteria, it can be officially released and commercialized for use in agriculture or other applications.

Statistical tools and software for AB-QTL analysis:

AB-QTL (Advanced Backcross-QTL) analysis is a statistical approach used to identify quantitative trait loci (QTLs) associated with complex traits in genetic mapping experiments. Below, I'll provide an overview of statistical tools and software commonly used in AB-QTL analysis, as well as their applications in linkage analysis, association mapping, and genome-wide association studies (GWAS) (Miles and Wayne, 2008) :

R/QTL: R/QTL is an R package designed for QTL mapping in experimental crosses. It provides a wide range of functions for genetic mapping, including the identification of QTLs, visualization, and significance testing.

MapQTL: MapQTL is a software package for QTL analysis that includes tools for interval mapping, composite interval mapping, and permutation tests for significance estimation.

Linkage analysis: Linkage analysis is used to identify genetic loci (QTLs) that co-segregate with the trait within a family or population. This method is particularly suitable for Mendelian traits. Software packages like MapQTL and R/QTL can be used for linkage analysis in AB-QTL studies. They allow for interval mapping and LOD score calculations to identify linked QTLs.

Association mapping (or Association Analysis): Association mapping is used to identify QTLs associated with a trait in populations of unrelated individuals. This method is useful for complex traits influenced by multiple genes. While association mapping is more commonly associated with GWAS (see below), some software like QTLbase and TASSEL can be adapted for association analysis in the context of AB-QTL.

Genome-Wide Association Studies (GWAS): GWAS is a powerful approach used to identify common genetic variants associated with complex traits. It involves testing genetic markers across the entire genome for association with the trait. Several software packages are available for conducting GWAS, including PLINK, GCTA-GWAS, TASSEL, and SNPTEST. These tools can handle large-scale genotyping data, perform statistical tests like logistic or linear regression, and correct for population structure and multiple testing.

Case Studies (Wang *et al.,* 2010)

Advanced Backcross-QTL (AB-QTL) analysis has been instrumental in crop improvement by identifying and characterizing quantitative trait loci (QTLs) associated with important agronomic traits. Here are a few case studies that showcase the successful application of AB-QTL analysis in different crops and highlight its impact on crop improvement:

A. Rice (*Oryza sativa*)

In rice, researchers used AB-QTL analysis to identify QTLs associated with drought tolerance. They developed advanced backcross populations by crossing drought-tolerant wild rice species (Oryza rufipogon) with cultivated rice varieties. Through AB-QTL analysis, they identified specific genomic regions linked to improved drought tolerance.

Impact: The identified QTLs have been incorporated into breeding programs to develop new rice varieties with enhanced drought tolerance, addressing the challenge of water scarcity in rice production.

B. Wheat (*Triticum aestivum*)

AB-QTL analysis has been applied in wheat to improve resistance to various diseases, such as wheat leaf rust. Researchers created advanced backcross populations by crossing wheat varieties with wild wheat relatives containing resistance genes. AB-QTL analysis helped identify and map the resistance QTLs.

Impact: The QTLs for disease resistance have been used in marker-assisted breeding to develop wheat varieties with improved resistance to leaf rust, reducing the need for chemical fungicides and increasing yield stability.

C. Maize (*Zea mays*)

In maize, AB-QTL analysis has been used to enhance grain yield. Researchers crossed maize inbred lines with teosinte, the wild ancestor of maize. Through AB-QTL analysis, they identified QTLs associated with increased grain yield and kernel size.

Impact: These QTLs have been used to develop maize hybrids with higher yield potential, contributing to food security and the economic well-being of farmers.

D. Tomato (*Solanum lycopersicum*)

AB-QTL analysis in tomato has been applied to improve fruit quality traits, such as fruit size and shelf life. By crossing cultivated tomato varieties with wild tomato species, researchers identified QTLs associated with these traits.

Impact: The QTLs have been used to develop tomato cultivars with larger, longer-lasting fruits, enhancing marketability and consumer preference.

E. Barley (*Hordeum vulgare*)

In barley, AB-QTL analysis has been employed to improve malting quality, a crucial trait for the brewing industry. Researchers created advanced backcross populations by crossing barley lines with wild barley accessions containing desirable malting characteristics.

Impact: The identified QTLs have been integrated into breeding programs to develop barley varieties with improved malting quality, leading to better beer production and economic benefits for barley growers.

Summary:

In summary, QTL analysis is a foundational component of modern crop improvement efforts. Its ability to identify and understand the genetic basis of important traits, coupled with its capacity to expedite breeding cycles and enhance trait selection, makes it an invaluable tool for developing improved crop varieties to address the evolving

needs of agriculture and society. Mapping QTLs in plant genomes is a powerful approach that bridges the gap between genotype and phenotype, providing valuable insights into the genetic architecture of quantitative traits and aiding in crop breeding efforts to develop more resilient and productive plant varieties. The genetic basis of complex traits involves the interaction of multiple genes, genetic variants, environmental factors, and gene-environment interactions. The study of complex traits is essential in fields such as genetics, agriculture, medicine, and ecology, as it sheds light on the inheritance patterns and mechanisms responsible for the continuous variation observed in these traits. Overall, the choice of statistical tools and software in AB-QTL analysis depends on the specific design of the study, the type of genetic data available (e.g., microsatellites, SNPs, or sequencing data), and the nature of the trait being studied. Researchers should select the most appropriate methods and tools based on their research objectives and data characteristics. The case studies illustrate the versatility and impact of AB-QTL analysis in crop improvement. By identifying and characterizing specific genomic regions associated with desirable traits, AB-QTL analysis has played a pivotal role in accelerating the development of new crop varieties with improved agronomic, nutritional, and quality attributes, ultimately benefiting farmers, consumers, and the agricultural industry as a whole.

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QUORUM SENSING IN ENDOPHYTES: SYMBIOTIC DYNAMICS AND AGRICULTURAL APPLICATIONS IN PLANTS

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Abstract:

This book chapter explores the intricate interplay between endophytes and wheat plants, focusing on the role of quorum sensing in shaping this symbiotic relationship. Endophytes, residing within plant tissues, contribute significantly to plant health and growth. Quorum sensing, a communication system among microorganisms based on signaling molecules, plays a pivotal role in orchestrating endophyte behaviour and its subsequent effects on wheat plants. This chapter explores AHL-based and AI-2-based quorum sensing mechanisms, emphasizing their impact on Gram-negative and Gram-positive endophytes. It provides an overview of the quorum sensing mechanism in endophytes, discussing its influence on plant growth promotion, stress tolerance, and disease suppression in wheat. The potential applications of understanding quorum sensing in endophytes for sustainable agriculture are also highlighted, emphasizing the promising prospects in optimizing plant growth and mitigating environmental challenges. Overall, the chapter underscores the importance of quorum sensing in endophytes as a potential tool to enhance agricultural practices and foster a harmonious plant-microbe relationship.

Keywords: Quorum sensing, endophytes, wheat, plant-microbe interaction, growth promotion, stress tolerance, disease suppression, signaling molecules, sustainable agriculture.

Introduction:

Endophytes are microorganisms, primarily bacteria and fungi, that reside within the tissues of plants without causing any visible harm to the host (Hardoim *et al.,* 2015). They form a unique symbiotic relationship with plants and often confer various benefits, including enhanced growth, stress tolerance, and disease resistance. Quorum sensing (QS) in endophytes is a crucial mechanism that governs communication and behavior within these microbial communities, influencing their interactions with wheat plants.

Quorum sensing mechanism

AHL-based quorum sensing:

AHL-based quorum sensing is the most common form of quorum sensing observed in Gram-negative endophytes and many other bacteria. AHLs, or acyl-homoserine lactones, are the signaling molecules used in this type of quorum sensing (Schaefer *et al.,* 2008). AHLs are synthesized by AHL synthases and are released into the extracellular environment. As the bacterial population density increases, AHLs accumulate in the surroundings. When AHLs reach a critical concentration, they bind to specific receptors, activating gene expression and triggering coordinated responses within the bacterial community (Fuqua *et al.,* 2001). These responses can include virulence factor production, biofilm formation, and other beneficial or pathogenic behaviours.

AI-2-based quorum sensing:

AI-2-based quorum sensing is a form of quorum sensing that uses a different type of signaling molecule, known as autoinducer-2 (AI-2). AI-2 is produced and detected by both Gram-negative and Gram-positive bacteria (Xavier & Bassler, 2005). LuxS is a key enzyme involved in AI-2 synthesis. AI-2 is released into the extracellular environment, and its concentration increases with the bacterial population density. Once a threshold concentration is reached, AI-2 is detected by receptors, and the signal is transduced to initiate changes in gene expression and cellular behavior (Surette *et al.,* 1999). AI-2 has been recognized for its role in inter-species communication, promoting interactions between different species of bacteria within the endophytic microbial community.

Understanding these two types of quorum sensing mechanisms in endophytes is crucial for unravelling the complexity of microbial interactions within plant tissues and their impact on plant health and growth.

Quorum sensing in gram-negative bacteria:

In Gram-negative bacteria, the most common signaling molecules involved in quorum sensing are acyl-homoserine lactones (AHLs) (Waters & Bassler, 2005). These molecules are produced by specific enzymes, AHL synthases, and are released into the extracellular environment. When the bacterial population reaches a certain density, the concentration of AHLs in the environment increases, enabling them to bind to specific receptors, often transcription factors, within the bacterial cells (Schuster *et al.,* 2004).

The AHL-receptor complex then activates or represses the expression of target genes, particularly those associated with virulence factors, biofilm formation, antibiotic

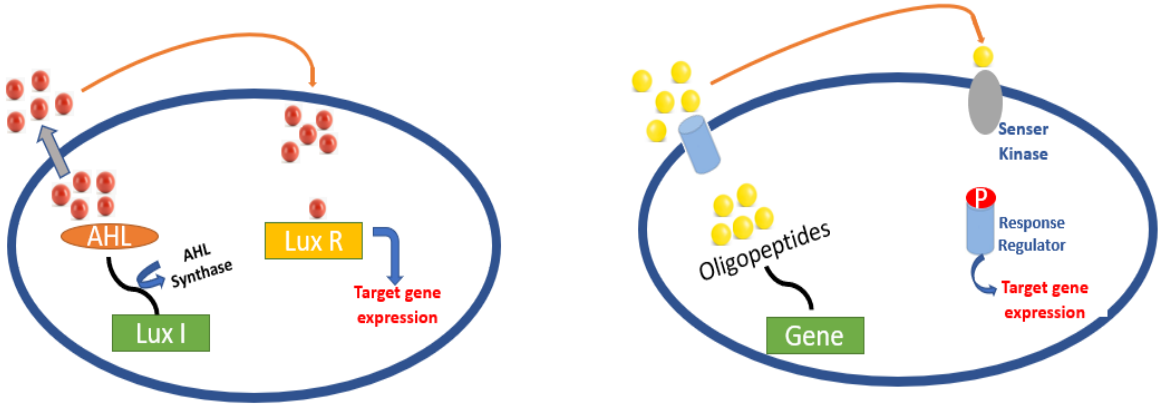
resistance, and other cooperative behaviours (Fuqua *et al.,* 1994). Examples of Gram-negative bacteria that utilize AHL-based quorum sensing include *Pseudomonas aeruginosa*, *Vibrio fischeri*, and *Escherichia coli* (Williams *et al.,* 2007).

Quorum sensing in gram-positive bacteria:

In Gram-positive bacteria, signaling molecules involved in quorum sensing vary and can include modified peptides, autoinducing peptides (AIPs), and other small molecules (Bassler & Losick, 2006). These molecules are generally sensed through two-component signal transduction systems or alternative intracellular pathways (Papenfort & Bassler, 2016).

When the bacterial population density increases, the concentration of these signaling molecules also rises. Once a threshold concentration is reached, these molecules bind to specific receptors, often histidine kinases or sensor kinases, initiating a signaling cascade that leads to changes in gene expression (Ng & Bassler, 2009). This altered gene expression regulates various processes like virulence, competence, sporulation, and biofilm formation (Novick & Geisinger, 2008).

Well-known Gram-positive bacteria utilizing quorum sensing include *Staphylococcus aureus*, *Streptococcus pneumoniae*, and *Bacillus subtilis*, each using distinct signaling molecules and mechanisms (Rutherford & Bassler, 2012).



Gram negative Bacteria **Gram positive bacteria**

Quorum sensing molecules

There are several known types of molecules involved in QS, with acyl-homoserine lactones (AHLs) being a prominent class.

PGPR/endophytes	Signal Molecules	Reference
Pseudomonas spp.	AHLs (N-acyl homoserine lactones)	Schikora *et al.,* 2016
Bacillus spp.	Cyclic lipopeptides (various)	Raaijmakers and de Bruijn, 2009
Azospirillum spp.	IAA (Indole-3-acetic acid)	Patten and Glick, 2002
Rhizobium spp.	Nodulation factors (NFs)	Oldroyd and Downie, 2008
Mesorhizobium spp.	Nodulation factors (NFs)	Oldroyd and Downie, 2008

Quorum sensing mechanism in disease prevention

Quorum sensing in endophytes significantly contributes to disease prevention by coordinating defence mechanisms and antimicrobial compound production. Additionally, it positively influences plant growth by modulating phytohormone levels and optimizing nutrient availability.

1. Biocontrol and quorum sensing: Endophytic bacteria use quorum sensing to synchronize their defence mechanisms, aiding in biocontrol against plant pathogens.

This orchestrated response enhances plant resistance, ultimately contributing to the overall health of the plant (Gupta *et al.,* 2023).

2. Antimicrobial compound production: Quorum sensing prompts endophytes to produce antimicrobial compounds, exhibiting potent inhibitory effects against pathogenic microorganisms. The precise regulation through quorum sensing optimizes the efficacy of these compounds in safeguarding the plant from infections (Li *et al.,* 2023).

Quorum sensing in plant growth

1. Phytohormone modulation: Endophytic bacteria influence plant growth by modulating phytohormone levels through quorum sensing. This regulation includes key hormones like auxins, cytokinin's, and gibberellins, impacting plant growth and development positively (Sharma *et al.,* 2022).

2. Nutrient uptake and availability: Quorum sensing helps endophytes enhance nutrient availability to plants by coordinating nutrient acquisition and mobilization. This optimization supports nutrient absorption, promoting overall plant growth and development (Zhang *et al.,* 2022).

Conclusion and future perspectives

Quorum sensing (QS) in endophytes emerges as a critical mechanism governing microbial behavior and interactions within the complex plant tissue environment. This

chapter has provided a comprehensive understanding of two main types of QS mechanisms, AHL-based and AI-2-based, shedding light on their role in Gram-negative and Gram-positive endophytes. Significantly, these QS mechanisms contribute to disease prevention and influence plant growth by modulating phytohormone levels and nutrient availability. Harnessing the potential of QS in endophytes holds promise for enhancing plant health, mitigating diseases, and optimizing agricultural productivity. Future research should focus on unravelling the complex interplay between quorum sensing, endophyte behaviour and plant growth.

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ENZYMES: CLASSIFICATION AND NOMENCLATURE

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Enzymes: The basis of life

Our current existence on earth depends on enzymes. Without enzymes, processes would have happened very slowly, and humans might have been waiting to evolve while lying down in some volcanic or hydrothermal vents. The first life on Earth is estimated to have existed around 4.2 billion years ago, and humans first appeared about 2.5 million years ago. The world was born with a huge bang about 20 billion years ago. This indicates that inorganic evolution proceeded at a far slower rate than organic evolution, which is directly related to the faster rate of biological reactions. Enzymes, which may sometimes accelerate reactions up to a million times faster, are the key to improving the pace of reaction in biological systems. Enzymes are essential to life as we know it and are capable of carrying out biochemical processes efficiently. They are also widely used in many different industries and represent a multi-billion dollar business. In our daily lives, we frequently utilize products that have been processed with industrial enzymes. This makes enzyme very interesting components of biology and their study 'enzymology' an essential subject in life sciences.

Enzymology:

The study of enzymes has been an autocatalytic intellectual activity. Apart from knowledge gained on their structure and function, the study of enzymes is a driving force in advancing our understanding of biological phenomena as diverse as intermediary metabolism and physiology, molecular biology and genetics, cellular signaling and regulation, and differentiation and development.

The confidence in our experience with enzymes is so strong that they have found applications in a variety of industries including food, pharmaceuticals, textiles, and the environment.

We encounter enzymes in every facet of biology and are forced to admire their exquisite roles. Enzymes were excellent models and earliest examples to understand protein structure-function. These include enzymes like hen egg white lysozyme, bovine pancreatic ribonuclease A (RNase A), trypsin, and chymotrypsin. A few of these were encountered during the study of digestive processes. Selectivity of proteases was exploited,

and they served as useful reagents to cleave and study protein structure. The field of molecular biology has benefited enormously from enzymatic tools to cut, ligate, and replicate information molecules like DNA and RNA. Metabolic and cellular regulation is unthinkable without involving enzymes and their response to various environmental cues. The complexity associated with life processes owes it largely to their catalytic versatility, exquisite specificity, and ability to be modulated.

Enzymes are biocatalysts and they catalyse the biochemical reactions both *in vivo* as well as *in vitro*. They are highly specific to its substrate and have great catalytic power, i.e., they enhance the rate of reaction tremendously without being changed. All enzymes are proteins with exception of some small group of catalytic RNA molecules called **ribozymes**. Like proteins, the molecular weight of enzymes ranges from about 2000 to more than one million Dalton. Enzymatic activity of proteinaceous enzymes may be affected depending on the conformational structure as well as its denaturation. There are many enzymes which require cofactors for their catalytic activity. The cofactor may be a complex organic molecule called coenzyme or it may be a metal ion such as Fe^{2+}, Mn^{2+}, Zn^{2+}, Mg^{2+}. An enzyme plus its cofactor is called **holoenzyme**. In such cases, the protein component in cofactor requiring enzyme is called **apoenzyme**.

Holoenzyme = Apoenzyme + Cofactor

Coenzyme

Coenzymes are small organic molecules that transport chemical groups from one enzyme to another. Some of these chemicals such as riboflavin, thiamine and folic acid are vitamins. Such compounds cannot be made in the body and must be acquired from the diet. The chemical groups carried include the hydride ion (H−) carried by NAD or NADP+, the acetyl group carried by coenzyme A, formyl, methenyl or methyl groups carried by folic acid and the methyl group carried by *S*-adenosylmethionine.

Since coenzymes are chemically changed as a consequence of enzyme action, it is useful to consider coenzymes to be a special class of substrates, or second substrates, which are common to many different enzymes. For example, about 700 enzymes are known to use coenzyme NADH.

Coenzymes are usually regenerated and their concentrations maintained at a steady level inside the cell. Coenzyme are organic compounds required by many enzyme for catalytic activity.

Coenzymes take part in catalysis transiently and are carriers of specific functional groups. Most of the coenzymes are derived from vitamins (organic nutrients required in small amounts in diet).

Some coenzymes and their precursor vitamins and their role

Coenzyme	**Precursor vitamin**	**Role in the catalytic reaction**
Biocytin	Biotin (vitamin B7)	Transfer of CO_2
Coenzyme B12	Vitamin B12	Transfer of an alkyl group
Flavin adenine dinucleotide (FAD)	Riboflavin (vitamin B2)	Transfer of electrons
Coenzyme A	Pantothenic acid (vitamin B3)	Transfer of acyl and alkyl group
Nicotinamide adenine dinucleotide (NAD)	Niacin (vitamin B5)	Transfer of hydride (:H-)
Pyridoxal phosphate	Pyridoxine (vitamin B6)	Transfer of amino group
Thiamine pyrophosphate	Thiamine (vitamin B1)	Transfer of aldehydes
Tetrahydrofolate	Folic acid (vitamin B9)	Transfer of one carbon group

Metal ions that serve as cofactors for enzymes

Metal Ions	**Enzyme name**
Fe^{2+} or Fe^{3+}	Catalase, peroxidase, cytochrome oxidase
Cu^{2+}	Cytochrome oxidase
Mg^{2+}	DNA polymerase
Mn^{2+}	Arginase
K^{+}	Pyruvate kinase
Mo^{2+}	Nitrogenase, nitrate reductase
Zn^{2+}	Carbonic anhydrase, alcohol dehydrogenase
Ni^{2+}	Urease

When a coenzyme or metal ion is tightly bound through covalent bond with the enzyme protein, it is called a prosthetic group.

Glorious past of enzymology

Enzymology had a glorious past, both in terms of recognition of the studies (several Nobel prizes were awarded) as well as commercialization (a large enzymology industry exists). The oldest known reference to the commercial use of enzymes comes from a description of wine making in the Codex of Hammurabi from ancient Babylon civilization around, 2100 BC. References from the writings of various ancient civilization from Babylon,

Rome, Greece, Egypt, China, and India suggest the use of microorganisms as enzyme sources for fermentation was widespread among ancient people.

Buchner's accidental discovery won him the 1907 Nobel Prize in chemistry. The next breakthrough in enzymology came in 1926 when J. B. Sumner from Cornell University, U.S.A., isolated, purified and also successfully crystallized the enzyme urease from jack bean. (Urease breaks down urea and produces ammonia and carbon dioxide). He found that the urease crystals are purely made of proteins and hence he reported that "enzymes are nothing but proteins". But his conclusion was vehemently opposed by the well-known German biochemist Richard Willstäter, (Nobel laureate), who insisted that enzymes are nothing but 'low molecular weight organic compounds' and the protein crystals that were found in the urease preparation could be 'impurities'. For their seminal contributions to enzymology, Sumner and Northrop were awarded Nobel Prize in 1935.

The above discoveries proved beyond doubt that enzymes are nothing but proteins and are found in all living cells. Therefore, it was imperative that only living organisms could make such sophisticated enzyme molecules. However, development of solid-phase peptide synthesis by R. B. Merrifield and his group in 1964 paved the way for laboratory synthesis of enzymes for the first time. The first enzyme that was assembled on a solid phase matrix was the pancreatic ribonuclease, which contains 124 amino acids. Interestingly, the laboratory-assembled enzyme exhibited identical properties as those of the natural enzyme. Therefore, this discovery added one more dimension to enzymology and led to the synthesis of tailor-made synthetic enzymes, which are known as synzymes. Later in 1969, Jenks, suggested that generating antibodies raised against a stable analogue of the transition state of the reaction that one wished tocatalyze, one could obtain antibodies with catalytic activity, and such antibodies were called abzymes. The discovery of new types of enzymes, viz., the restriction enzymes and ligases in 1960s paved way for the modern discipline, the recombinant DNA technology. The discovery of restriction endonucleases by W. Arber, D. Nathan, Peter Lobbund and Dale Kaiser's work on enzymatic joining of DNA fragments led to the construction of the first recombinant DNA molecule in 1980s. Arber and Nathan were awarded Nobel Prize for their discovery of the restriction endonucleases in 1978. Thus, the successful application in construction of recombinant DNA molecules paved the way for today's new and vibrant branch of biology, the Biotechnology.

The belief that "all enzymes are proteins" was shattered in 1982 when Thomas R. Cech and Sidney Altman independently discovered that certain RNA molecules also exhibited catalytic properties like enzymes. The discovery of catalytic RNAs forced proteins

to relinquish their status as the only molecules that can catalyze chemical reactions in living cells. These ribonucleic acid enzymes are called as ribozymes. For this important discovery in enzymology, they were also awarded Noble Prize in 1989.

Nomenclature and classification of enzymes

The word enzyme (meaning is yeast in Greek), first used by Kuhne in 1877, is now well accepted to describe a biological catalyst. Majority of enzyme names today carry the suffix "-ase" as recommended for all enzyme names by Duclaux in 1898. Proteolytic enzymes are a significant exception to this generally accepted norm. Some of them have retained the older tradition of usually ending with "-in," for example, trypsin, chymotrypsin, papain, and subtilisin.

In order to have a systematic study and to avoid ambiguities considering the fact that new enzymes may also be discovered, International Union of Biochemistry (I.U.B.) in 1964 has adopted classification of enzymes depending on the type of reactions they catalyze. According to this commission, all enzymes are classified into 6 major classes.

Classification of enzymes adopted by I.U.B.

No. Class	Class name	Type of reaction catalyze
1	Oxidoreductases	Oxidation-reduction reactions (transfer of electrons)
2	Transferases	Transfer of groups
3	Hydrolases	Hydrolytic reactions (transfer of functional groups to water)
4	Lyases	Addition or removal of groups to form double bonds
5	Isomerases	Transfer of groups within molecules to yield isomeric forms
6	Ligases	Condensation of two molecules coupled through ATP hydrolysis

Oxidoreductases: catalyze oxidation/reduction reactions which generally involve the transfer of electrons. Examples are oxidases or dehydrogenases.

Transferases: transfer a functional group (e.g. a methyl or phosphate group) and these generally involve the transfer of a radical. Examples are: transglycosidases, e.g. of monosaccharides; transphosphorylases and phosphomutases, e.g. of a phosphate group; transaminases, e.g. of an amino group; transmethylases, e.g. of a methyl group; and transacetylases, e.g. of an acetyl group.

Hydrolases: catalyze the hydrolysis of various bonds. The hydrolase reaction generally involves addition or removal of water. Examples are: hydrolases, including esterases, carbohydrases, nucleases, deaminases, amidases and proteases; hydrases such as fumarase, enolase, aconitase and carbonic anhydrase.

Lyases: cleave various bonds by means other than hydrolysis and oxidation. This reaction involves the splitting or forming a C=C bond. Examples are desmolases.

Isomerases: catalyze isomerization changes within a single molecule and involve changing the geometry or structure of a molecule. An example is glucose-isomerase.

Ligases: join two molecules with covalent bonds.

Isozymes

Isozymes are enzymes that differ in amino acid sequence but catalyze the same chemical reaction. Isozymes usually have different kinetic parameters, or are regulated differently. They permit the fine-tuning of metabolism to meet the particular needs of a given tissue or developmental stage. For example, a glycolytic enzyme, hexokinase exists in four isozyme forms in various tissues. Similarly, lactate dehydrogenase (LDH), involved in anaerobic glucose metabolism has two isozyme forms in human, one is present in heart and the other is found in skeletal muscles.

Enzyme active site

The active site of an enzyme is the part of the enzyme where substrate molecules bind and a chemical reaction takes place. The active site is made up of amino acid residues that establish temporary bonds with the substrate (binding site) as well as residues that catalyse that substrate's reaction (catalytic site). Active site has a three-dimensional structure since it consists of portions of a polypeptide chain. Various non covalent bonds involved in enzyme substrate binding are electrostatic interactions, hydrogen bonds, Van Der Waals forces and hydrophobic interactions. The active site often comprises non polar environment which facilitates the binding of substrate and the catalysis.

Fischer's Lock and Key Model

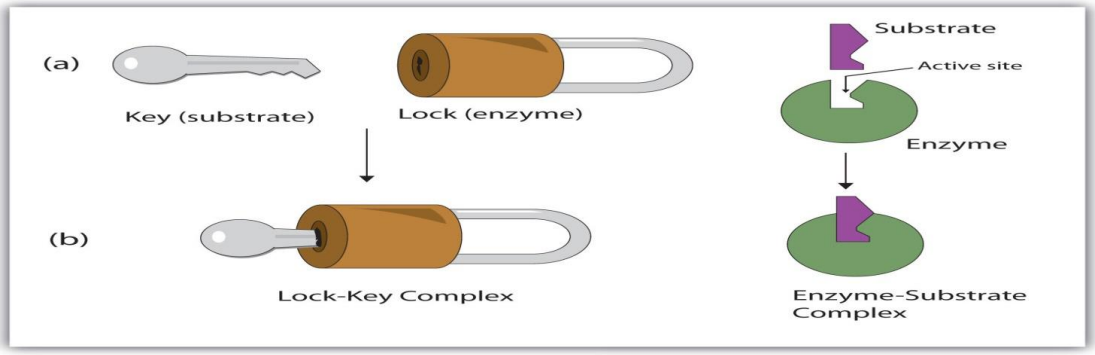

Figure 1: Interaction between an enzyme and its substrate according to lock and key model

In 1894, the introduction of Lock and Key Model for the substrate and enzyme interaction was proposed by Emil Fischer. According to this model, complementary structural features are present between enzyme and substrate, and the active site is pre-

shaped to fit the substrate. The substrate can fit into its complementary site on the enzyme as a key fits into a lock. This results in the formation of an enzyme-substrate complex (Fig.1).

Koshland's Induced Fit model

Daniel Koshland in 1958 proposed Induced Fit Hypothesis. He suggested that the structure of a substrate may be complementary to that of the active site in the enzymesubstrate complex but not in the free enzyme. The interaction between the substrate and the enzyme induces conformational changes in the enzyme which aligns the amino acid residues or other groups for substrate binding, catalysis, or both. The relationship between a substrate and an active site resembles hand and woollen glove. During interaction, the structure of one component, i.e., substrate or hand remains rigid and the shape of the second component, i.e., active site or glove flexible to become complementary to that of the first (Fig.2).

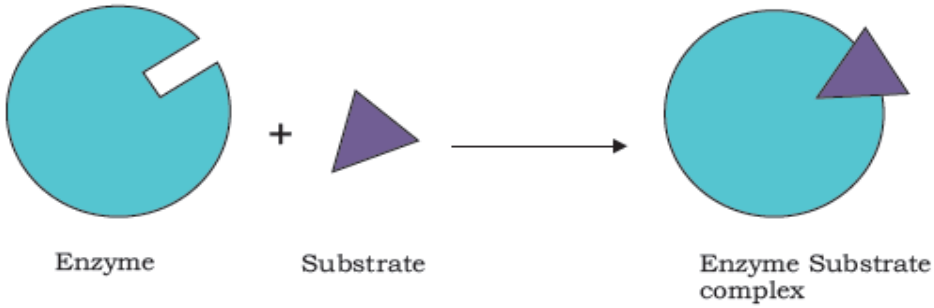

Figure 2: Interaction between an enzyme and its substrate according to induced fit model

Specificity of enzymes

One of the most relevant and also intriguing properties of enzymes is their specificity. Some enzymes exhibit absolute specificity. This means that these enzymes catalyze only one particular reaction. Other enzymes will be specific for a particular type of chemical bond or functional group. In general, there are four distinct types of specificity:

Absolute specificity: highly specific enzymes catalyze only one reaction.

Group specificity: group specific enzymes act only on molecules that have specific functional groups, such as amino, phosphate or methyl groups.

Linkage specificity: such enzymes act on chemical bonds of certain nature, regardless of the rest of the molecular structure.

Stereochemical specificity: stereospecific enzymes act only on a particular steric or optical isomer and not on their isomeric counterparts.

The specificity of enzymes is determined by complementary shape, charge, hydrophilic/hydrophobic characteristics of the substrates and their three-dimensional

organization. The three-dimensional interaction has been described in various interaction models.

Factors affecting enzyme activity

Rate of enzyme catalysed reactions is influenced by changing the environmental conditions. The important factors that influence the velocity of enzyme catalysed reactions are temperature, pH, substrate concentration and modulators.

(1) Temperature

The rate of an enzyme catalysed reaction increases with the increase in temperature up to a maximum and then falls. When a graph is plotted between temperature versus enzyme activity, a bell-shaped curve is obtained. The temperature at which the maximum rate of reaction occurs is called the enzyme's optimum temperature. The optimum temperature is different for different enzymes; but for most of the enzymes it is between 40°C-45°C. Majority of enzymes in the human body have an optimum temperature of around 37°C (98.6°F) and are denatured or degraded at extreme temperatures. However, few enzymes like Taq DNA polymerase present in thermophilic bacteria, *Thermus aquaticus,* venom phosphokinase and muscle adenylate kinase are active even at 100°C.

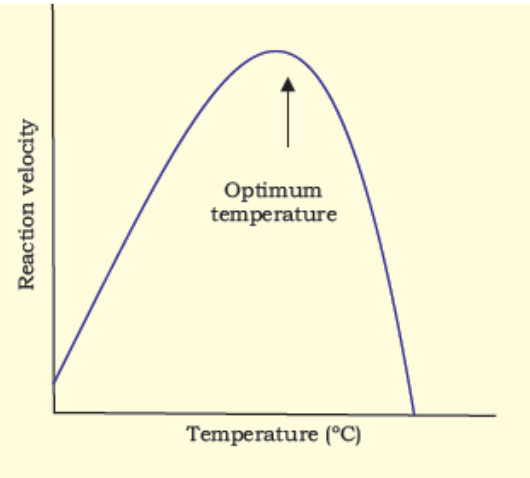

Figure 3: Effect of temperature on enzyme activity

(2) Hydrogen Ion Concentration (pH)

Enzyme activity is also affected by pH. A plot of enzyme activity against pH results in a bell shaped curve (Fig. 4.4). Each enzyme has its unique optimum pH at which the rate of reaction is greatest. The optimum pH is the pH at which the activity of a particular enzyme is at maximum. Many enzymes of higher organisms show optimum reaction rate around neutral pH (6-8). However, there are several exceptions such as pepsin (pH 1-2), acid phosphatases (pH 4-5) and alkaline phosphatases (pH 10-11). Below and above the optimum pH, the enzyme activity is much lowered and at extreme pH, the enzyme becomes totally inactive.

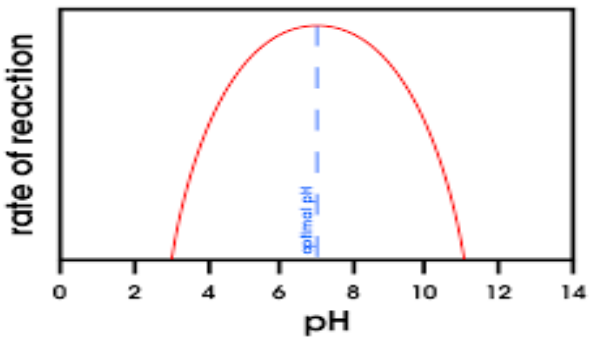

Figure 4: Effect of pH on enzyme activity

3. Substrate concentration

The substrate concentration also influences enzyme activity. As the substrate concentration increases the rate of reaction also increases. This is because the more substrate molecules will interact with enzyme molecules, the more products will be formed. However, after a certain concentration, further increase in substrate concentration will have no effect on the rate of reaction, since the substrate concentration will no longer be the limiting factor. At this stage, enzyme molecules become saturated and work at their maximum possible rate.

Mechanisms of enzymes

Enzymes can act in several ways, whereby each enzyme lowers the energy needed for the reaction to occur or to proceed. These mechanisms are described briefly as follows:

- Lowering the activation energy by creating an environment in which the transition state is stabilized. This can be achieved by binding and thus stabilizing the transition-state conformation of the substrate/product molecules.
- Lowering the energy of the transition state, but without distorting the substrate, by creating an environment with the opposite charge distribution to that of the transition state.
- Providing an alternative pathway. For example, temporarily reacting with the substrate to form an intermediate enzyme–substrate (ES) complex, which would be impossible in the absence of the enzyme.
- Reducing the reaction entropy change by bringing substrates together in the correct orientation to react. Considering an energy effect (_*H*‡) alone overlooks this effect.

Inhibitors

Enzyme inhibitors are substances which alter the catalytic action of the enzyme and consequently slow down, or in some cases, stop catalysis. Most theories concerning inhibition mechanisms are based on the existence of the ES complex. Substrate inhibition will sometime occur when excessive amounts of substrate are present.

There are three common types of enzyme inhibition – competitive, non-competitive and substrate inhibition. Besides these inhibitor types, a mixed inhibition exists as well.

1. Competitive inhibitors

In competitive inhibition, an inhibitor that resembles the normal substrate binds to the enzyme, usually at the active site, and prevents the substrate from binding. At any given moment, the enzyme may be bound to the inhibitor, the substrate, or neither, but it cannot bind both at the same time. Competitive inhibition is interruption of a chemical pathway owing to one chemical substance inhibiting the effect of another by competing with it for binding or bonding.

2. Non-competitive inhibition

Non-competitive inhibitors are considered to be substances which, when added to the enzyme, alter the enzyme in a way that it cannot accept the substrate. Noncompetitive inhibition, a type of allosteric regulation, is a specific type of enzyme inhibition characterized by an inhibitor binding to an allosteric site resulting in decreased efficacy of the enzyme. An allosteric site is simply a site that differs from the active site- where the substrate binds.

3. Substrate inhibition

Substrate inhibition is the most common deviation from Michaelis–Menten kinetics, occurring in approximately 25% of known enzymes. It is generally attributed to the formation of an unproductive enzyme–substrate complex after the simultaneous binding of two or more substrate molecules to the active site.

Summary

- Enzymes are catalysts that catalyse the biochemical reactions in the living system.
- Each enzyme has an active site into which the substrate molecule fits precisely. The 'lock and key' hypothesis postulated that the substrate fits precisely into the lock of the enzyme. This hypothesis has now been modified.
- The modern 'induced fit' hypothesis does not regard active site as a rigid structure but a flexible one, which modifies its shape to fit precisely the substrate molecule.
- Various factors like temperature, pH, substrate concentration and presence of inhibitors and activators influence the rate of enzyme catalysed reactions.
- Enzymes lower the activation energy of the reaction they catalyse.
- Simple single substrate enzyme catalysed reactions can be described by Michaelis-Menten kinetics which has a hyperbolic graph in terms of substrate concentration and initial velocity.

- Enzymes are also affected by the presence of inhibitors, like competitive, non-competitive and uncompetitive inhibitors, which slow down the rate of reaction or stop it completely.

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GENETICALLY MODIFIED FOODS – NEW WAY TO INCREASE AGRICULTURAL PRODUCTION

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Abstract:

Growing world population has a negative impact on food supply, and low agricultural yields are a result of pests and unfavorable weather and climatic circumstances. The development of genetically altered crops that are pest and drought resistant has offered a viable approach for increasing food supply. All living things are being subjected to a massive worldwide genetic experiment with the growth of genetically modified (GM) crops on millions of hectares of land and their introduction into our food supply. Given the rapid rate of new developments in the production of genetically modified crops, it is difficult for consumers, farmers, and governments to agree on a precise vision for the future of the global food supply. Although some people believe that genetically modified foods should not be trusted, genetically modified food crops have been hailed by some as a solution to the world's hunger problems. Genetically modified foods have been linked to a number of negative traits, including a propensity to increase antibiotic resistance, changes to the natural quality of food, a propensity to be poisonous, and a propensity to be allergic.

Keywords: GM foods, physical methods, *A. tumefaciens*, pesticide resistance.

Introduction:

The economic foundation of either our nation or India is agriculture. Humans engage in agriculture to produce food, fiber, fuel, furniture, and fodder. Production of crops and livestock for human consumption and employment constitutes the science, the arts, and the business of agriculture. The word "agriculture" is derived from the Latin word "Agerculturura," which directly translates to "cultivation of land" (ager = a field; culture = to cultivate). The practice of cultivating plants from the soil is referred to as agriculture. It means that man has made a deliberate and determined attempt to use the soil to his

advantage and goes beyond simple land tilling. It encompasses all actions taken by people to promote the rapid and improved growth of plant and animal products for the benefit of people. All facets of crop production, including horticulture, livestock raising, fisheries, forestry, etc., are covered by the applied science of agriculture. It encompasses knowledge of how to run a farm with skill as part of its definition as an art. Utilizes all technologies created on the basis of scientific knowledge, including crop breeding, production methods, crop protection, economics, etc. in order to increase yield and profit. In order to produce food, feed, fiber, and fuel, agriculture as a business seeks to maximize net return by managing land, labor, water, and capital while utilizing information from multiple sciences.

World population vs current mode of food production

Between 2009 and 2050, the global population is projected to increase by more than a third, or 2.3 billion people. This is a far slower rate of growth than that observed during the previous four decades, when it increased by 3.3 billion, or more than 90%. This expansion is anticipated to occur almost exclusively in emerging nations. According to projections, urbanization will continue to accelerate, reaching 70 percent of the world's population in 2050 (up from 49 percent today), while rural populations will actually decline after peaking in the following ten years. According to the Food and Agricultural Organization of the United Nations (FAO), by 2050, there would need to be a 70% increase in global food production. The FAO recommends current mode of food production in emerging countries should be doubled where the population is growing more quickly. It is anticipated that the present food production would be under tremendous strain from the growing population. New agricultural technology practices provide the chance to boost food production. Seeds that have been genetically altered (GM) represent a substantial advance in the production of agricultural crops. Genetically modified (GM) seeds are those that have had certain traits added, such as insect or herbicide resistance respectively. Some farmers have embraced the technology rapidly. For farmers that have trouble applying pesticides and herbicides, the technology could be more suitable. For farming locations that are difficult for tractors to reach, near bodies of water, or in areas with strong winds, GM foods may be an excellent option.

The potential advantages of genetically modified food are:

1. Food that is more nourishing
2. Plants that are resistant to disease and drought need less environmental resources (such as water and fertilizer).

3. Lessen reliance on insecticides
4. Increase the availability of food with lower prices and longer shelf lives.
5. Animals and plants grow more quickly
6. Food with more enticing characteristics, such as potatoes that use less oil when frying
7. Foods can be prescribed as medicines or utilized as vaccinations.

Genetically modified foods

Foods made from genetically modified organisms (GMOs), specifically genetically modified crops, are referred to as genetically modified foods (GM Foods/Biotech Foods). Foods that have undergone genetic modification have had foreign genes put into their DNA. Genetic engineering techniques have incorporated precise alterations into the DNA of GMOs. According to WHO (World Health Organization), GM crops are modified crops in which the genetic material (DNA) has been changed in a way that does not happen normally through mating and/or natural recombination. These organisms can be plants, animals, or microbes. The first step in creating GM crops is to identify the desired gene and separate it from the host organism. Using laboratory techniques, the gene is inserted into the crop plant's DNA based on agrobacterium or gene gun techniques. Researchers must insert the gene(s) encoding for certain features into a plant cell in order to produce GM food, and they must then regenerate a plant through tissue culture.

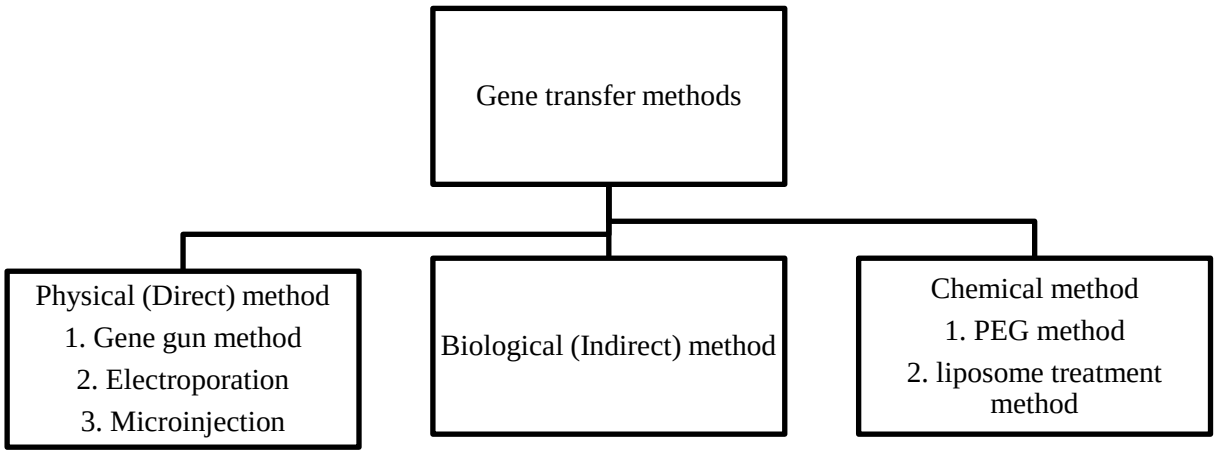

Figure 1: Different gene transfer methods

Physical methods

A) Gene gun method (particle bombardment):

The direct physical approach of delivering genes is known as the "gene gun method" or "particle bombardment." This technique involves firing the coated foreign object into the cell. With the use of a helium impulse, the foreign DNA is deposited onto high-density gold

or tungsten microcarriers and propelled into the target at a high speed. This allows it to pass through the cell wall and cell membrane. This approach is simple, quick, and adaptable.

B) Electroporation:

Electroporation is the process by which electrical current is given to a cell membrane to create pores on its surface for the transfer of genetic material. The typical voltage is between 100 and 150 volts. Cell burst could happen if it rises above this level. Lipid bilayer refers to the two layers of lipids that typically make up cell membranes. The gene of interest enters the cell through holes created when an electric current flows through them. The repair process is initiated and completed. Following it, replication occurs, resulting in the formation of new features.

C) Microinjection:

Through the use of delicate glass micropipettes and microinjections, a foreign gene is delivered into the host cell (egg, oocyte, or embryonic cell) during this operation. Since the desired gene is directly injected into the nucleus, this approach is more effective.

However, Microparticle bombardment (gene gun) is the method that delivers foreign DNA the most often. The DNA coated on gold or tungsten microparticles are transported at high velocities inside the targeted tissues, such as embryonic tissues from the seed. DNA may also be delivered into plant cells by electroporation and microinjection. However, particle bombardment continues to be more efficient.

Biological method (Indirect method)

A) Agrobacterium mediated gene transfer:

Exogenous gene insertion into plant cells entered a new era with the introduction of *Agrobacterium tumefaciens*. Plants that have been infected by the soil bacterium *A. tumefaciens* develop a gall at the crown. The bacteria really change the plant's genome, causing the cells to multiply and allowing the plant to create altered amino acids as a specialized food source for itself. The bacteria are able to insert genes because they have a "Ti-plasmid" (tumor inducing plasmid). This method involves the use of vectors, restriction enzymes (to cut the DNA fragments at specific point), DNA ligase (to attach the DNA fragments). Here the commonly used vector (i.e Ti-plasmid) is taken from the bacterium *A. tumefaciens.*

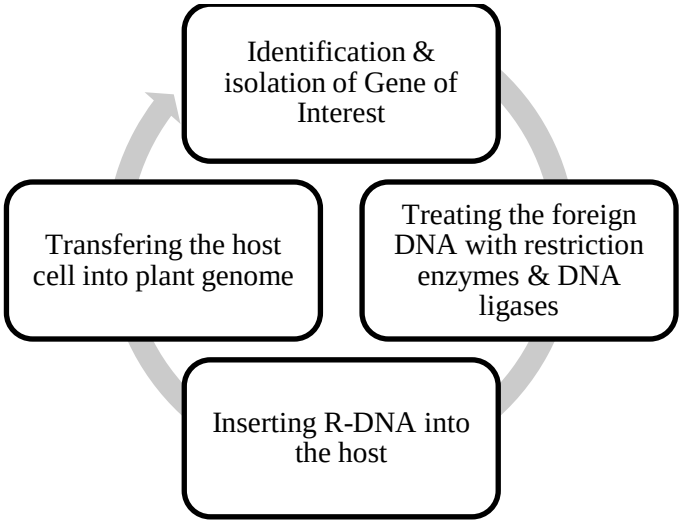

Figure 2: Steps involved in Agrobacterium mediated gene transfer

Chemical method

A) Liposome treatment:

Since liposomes are bilayer molecules, the lipid solution and DNA are combined here, and the DNA is subsequently trapped by the liposome. DNA is easily linked to the cell membrane via bilayer liposomes. When a DNA-loaded liposome is introduced to a host cell, the host cell membrane fuses with the liposome, allowing the gene of interest to be incorporated into the necessary plant or animal cell genome.

B) Polyethylene glycol (PEG) mediated gene transfer:

PEG weakens and makes the host cells' cell membrane permeable in the presence of divalent cations (Ca2+). Thus, the foreign DNA molecule penetrates the host cells' nucleus and interacts with the genome there. This technique is used and only appropriate for protoplast.

Table 1: List of approved GM crops

	GM Traits	**GM Crops**
1.	Antibiotic resistance	Apple
2.	Viral resistance	Papaya, squash
3.	Delayed ripening, antibiotic resistance	Melon, tomato
4.	Coleopteran insect resistance, blackspot bruise resistance	Potato
5.	Glyphosate herbicide resistance	Wheat
6.	Herbicide resistance	Maize, rice, cotton, canola, soybean, chicory, tobacco.
7.	Insect resistance	Cotton, tomato, potato, brinjal
8.	Increased beta-carotene	Golden rice

Advantages of using GM foods

1. Increased Crop production: Disease and rest resistance play a significant part in agriculture science and are mostly done to increase crop production. This contributes to keeping the worldwide demand for food steady.
2. Reduce Pesticide Use: Using fewer pesticides on crops that are resistant to them helps to lessen environmental issues.
3. Economical: Farmers saved money by using less pesticides and treatments as a result of the development of resistant plants, which increased their profitability.
4. Improved Food Quality and Safety: The use of pesticides improved the safety of food products by leaving behind fewer chemical residues, thereby improving consumer health.
5. Sustainable Agriculture: By minimizing environmental harm and lowering the need for resource-intensive procedures, disease- and pest-resistant crops support sustainable agriculture.
6. Food Security: Agriculture science helps to improve food security and lowers the likelihood of food shortages by defending crops against pests and diseases.
7. Lessened Environmental Issues: Less soil and water contamination from pesticide use helps to preserve ecosystems and biodiversity.
8. Global Agricultural Stability: Crops that are resistant to pests and disease contribute to the stability of agricultural systems by lowering the susceptibility of food production to outside influences.

Issues related to GM foods

Genetically modified (GM) food raises a number of ethical questions, such as:

1. Environmental problems:

It's possible for GM crops to crossbreed with wild kinds, which could have an impact on ecosystems. Concerns exist over how GM crops will affect creatures that are not their intended targets.

2. Human health:

Safety is crucial when it comes to eating gm foods, especially. The right of the consumer is another foundation for GM product labeling. Additionally, there is misunderstanding surrounding the product's labeling. Toxicity, allergenicity, and genetic hazards are the three main health problems that could be linked to GM food. The inserted gene and its expressed proteins as a whole, the secondary or pleiotropic effects of the gene-

expression products, and the potential disruption of endogenous genes in the altered organism are three potential causes of these.

3. Food safety:

When compared to conventional crops, gm crops boost yield and aid in pest resistance. However, they may have an adverse effect on small-scale farmers who may not be able to afford teg seeds, which may result in poverty and other economic problems.

4. Cultural and moral principles:

Cultural, religious, or ethical ideas about environment, food, and technology may be in opposition with GM foods.

5. Long-term effects:

There are ethical questions concerning the potential long-term effects of GM crops on the environment and human health.

It is a difficult challenge to balance the potential advantages of GM food, such as higher agricultural yields and less pesticide use, with these moral concerns.

Conclusion:

There is no evidence, according to science, that eating genetically modified food can harm a person's health. Despite the fact that numerous academic works have outlined scientific arguments for why eating foods that have undergone genetic modification may be dangerous, there are either few or no reported instances of genetically modified foods having a negative impact on humans. It is important to recognize the power of genetic modification because future study on the long-term impacts of genetically modified foods on people will be quite interesting. We are unable to go back in time and limit agriculture to practices that were created to feed a much smaller population. We will need to nearly double current production once more by 2025. This growth will not be possible until farmers everywhere have access to cutting-edge biotechnology innovations that can boost the yields, dependability, and nutritional value of our staple food crops as well as the existing high-yield crop production techniques. The sooner common sense is introduced into the discussion of agricultural science and technology, the better. A groundbreaking technology in the agriculture sector are genetically modified seeds. There is no doubt that these seeds also have a lot of potential advantages. Farmers should, however, accept new technology in an uninformed manner to make the world free from food scarcity in the future.

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AGRICULTURAL IN INDIA: A SHORT REVIEW

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The "Agriculture" word is derived from two Latin word "Agricultura" from "Ager field" and "cultura" "Cultivation", or growing agricltura is also known as "Commercial Grain Farming". Agriculture can be called the backbone of India's economic system because two-third of Indian Population is engaged in the cultivation of land. Agriculture not only help to feed large population but it also supports the principal manufacturing industries with raw materials.

Agricultural Science is a multidisciplinary field of biology that encompasses the part of exact natural economic and social science that are used in the practice and understanding of agriculture. It deals food and fiber production and processing. The technologies of soil cultivation, crop cultivation and harvesting animal production and processing of plant and animal products for human consumption and use are include in it.

Agriculture is an evolutionary process that consist of series of activities like production of food, fibers, and feed and raising of domesticated animals to fulfill the demands of population, agriculture is he art and science of cultivating the soil growing crops and raising livestock production, agriculture fisheries and forestry for food and nonfood products. It is the science and practice of growing crops by cultivation of the land. Agriculture can be called a primary activity which include the growing of vegetables, fruits, crops, flowers and also rearing livestock. Agriculture involves the various systematic use of growing crops that help in the production of food which is of the same kind of plant that are grown at a production place. Agriculture helps in the production.

Agriculture is the key to development in the area of human civilization. Agriculture has a vital role in the development of various industries especially agro based industries such as textile, sugar tea etc. Because of its complementing nature agriculture is consider as important sector in economic development. It also helps on the economy of the country. Agriculture plays a vital role in our life, without agriculture the existence of human being is not possible as it is the main source of our food, supply to sustain n earth and it also helps to grow our economy across the world.

India is an agricultural country. It has a great contribution to the Indian economy. Agriculture is the backbone of our country's economy. It is the main traditional occupation of our country. India produces both Kharif and Rabi crops. The main crops produced in India are Rice, Wheat, Maiz, Jute, Sugarcane others. Cereals, pulses, spices, cotton, tea, coffee etc. It provides food. Raw materials for industries and some product for export. It accounts for about 25% of the gross domestic product. Nearly two-third of its population depends directly on agriculture for livelihood. Agriculture is the main stay of India's economy. It is the primary activity of its nation. It provides employment opportunity to the rural agricultural as well as non-agricultural labors. It is the source of food and fodder. It also plays an important role in international business in import and export activities. Agriculture contributes to the marketable surplus with the development of a country more people get engaged in the non-agriculture sector like mining, manufacturing etc.

Agricultural innovation can help India eat emission, improve energy, security and boost farmer's income India's agricultural sector play a crucial role in the country's food security, energy security and de carbonization goals. Indian agriculture is multifaced with horticulture and animal husbandry.

Contributing to over 60% of India's agriculture GDP India is the largest milk producer rank 2$^{nd\ in}$ vegetables and fruits 3rd in fish, egg and poultry production in the world. Agriculture is the main stage of Indian economy as about 60% of our population depends directly or indirectly on agriculture. It provides raw materials to the industries India earn foreign exchange by exporting agriculture products.

India at present is also one of the largest producers of Wheat and Rice as also livestock and poultry. The agriculture sector is a central pillar of the Indian economy. A large portion of India is full of highly fertile land. India is a decently populated country and hence needs food grain production on a large scale. The mansoon climate of India is highly favorable for farming.

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VERTICAL FARMING- CONCEPT AND ITS SCOPE IN FUTURE

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Abstract:

With ever increasing population across the globe per capita availability of natural resources is shrinking at a faster pace. Decrease in per capita availability of land is posing a serious threat to food security and on the other hand changing climate is making crop production more vulnerable to pests and diseases. To meet the food demands of the world simultaneously overcoming the negative impact on environment is possible with vertical farming. Hydroponics, aquaponics and aeroponics are the three different systems of vertical farming where crops are grown in nutrient rich solution. Not all the crops that are grown in open fields can be grown under vertical structures, there are certain specifications like short stature, short production cycle and high value crops can only be grown here. Vertical farming has advantages of reducing food miles, supplying fresh foods and generating employment in urban space and it also has certain disadvantages like availability of space in urban areas, Waste disposal and Uninterrupted power source.

Keywords: Aeroponics, Aquaponics, Food miles, Hydroponics, Vertical farming.

Introduction:

An emerging global problem is the long-term decreasing stock of agricultural land per capita. Statistics on future growth of the world population from the United Nations Food and Agriculture Organization (FAO) reveal that arable land per person is projected to decrease by 2050 to one-third of the amount available in 1970 (FAO 2016). This decline is forecasted to continue due to the effects of climate change, the increasing geographic extent of drylands, the reduction in fresh water supply, and population growth. Planet is running short of farmland to feed a growing number of people. A more complete list of prominent threats to the future supply of arable land would also include: climate change, declining fisheries (prompting a greater food burden on land-based products), increasing urbanization, rising costs of agribusiness (e.g., fertilizers, fuel, pesticides), rapidly increasing population, soil depletion, and degradation from over-farming and poor production practices.

There is increasing realization that primary producers such as Australia are too small to be the food bowl for Asia as current agricultural production would feed only about 60 million people. At the present time, scaling up to provide for the region's entire population of 3 billion people is physically impossible. The comparative economic advantage of Australia is in providing so-called clean, green, and gourmet (CGG) foods for the rapidly growing middle and upper classes in Asia, particularly China, while expanding the current volume of production as fast as possible.

Interest in vertical farming gained traction following publication of the book by Despommier (2010) who argued that the benefits of indoor greenhouse farming could be multiplied greatly by building high-rise buildings in urban environments. In the CGG food category, pilot farms have been established in various cities around the world including London, New York, Singapore, and Tokyo. In particular, China, Japan and Israel are devoting resources to indoor factory farming due to issues related to climate, pollution, and urbanization. The advantages and disadvantages of this approach have been the subject of continuing analysis at the industry level but more attention is needed with respect to planning, policy, and economics.

Controlled environment agriculture

The vertical farming model is essentially an indoor farm based on a high-rise multi-level factory design. Typical features include innovative use of recycled water augmented by rainwater or water from a desalination plant, automatic air-temperature and humidity control, solar panel lighting and heating, and tunable 24-hour LED illumination. The LED equipment can be controlled throughout a growing season to emit a programmed spectrum of light that is optimal for photosynthesis for different types of crops. When coupled with regulation of temperature and humidity, the effects of seasonality can be minimized or eliminated. An indoor vertical farm may not even need soil if hydroponics is used. This cultivation technique involves growing plants in a soil-free culture with nutrient solutions. The plants are suspended in a medium, such as rock wool or perlite, and provided with nutrients, or the roots are directly bathed in the nutrient liquid using the nutrient-film technique. Air conditioning provides a constant flow of air which can be enriched with carbon dioxide (CO_2) to further advance plant growth and development. Both ambient and nutrient temperatures can be held at specific levels that optimize the rate of plant growth. Any nutrients and water not absorbed by the roots can be recycled rather than lost to the system. The approach is consistent with CGG food production. It can be used to grow a wide

range of crops, pharmaceuticals, or herbs. A variant of hydroponics is aeroponics which involves spraying the roots of plants with atomized nutrient solutions or mists. There is reduced need for fertilizers, herbicides, and pesticides if there is effective isolation from a harsh external climate. Such a factory would essentially eliminate common constraints and risks to productivity, including heat and drought, pests, seasonality, and transportation costs from remote locations. Volatility in markets can be addressed because production can be planned according to demand. There are also implications for future food security and sustainability in the face of climate change and diminishing land and water resources.

Different Types of Vertical Farming Systems

Hydroponics

This technique for vertical farming suggests growing plants without the need for soil. Hydroponic systems submerge plant roots into liquid solutions with different nutrients. Instead of using soil, materials such as gravel and sand are used as substitutes for supporting the plants' roots. This allows macronutrients, or the nutrients required in large amounts, to be taken and focused on by plants. Advantages of hydroponic farming include increasing the production of crops in an area and decreasing the amount of water used for the plants overall.

Aquaponics

Aquaponics combines aquaculture and hydroponics. Aquaculture refers to fish farming, and hydroponics is growing plants without soil. Aquaponics takes hydroponics to the next level by integrating earthly plants' production while using aquatic organisms to help their growth. In a closed-loop system (or a system that sustains itself without outside organisms), the plants' environment will mimic their natural habitat. Combining natural aquatic organisms without the use of soil helps the plants focus on the intake of natural materials and nutrients. The main focus of aquaponics is to produce more slower-growing crops while including some more aquatic organisms. Since this still utilizes nutrients but at a reduced rate, it isn't used as often as other methods of vertical farming.

Aeroponics

This system type was utilized best by NASA to find an efficient way of producing crops in unconventional circumstances. NASA mainly sought out this technique to see how easy it was to grow plants in space in the 1990s. Aeroponics is unique because it uses no soil or aquatic organisms to grow. Instead, the absence of a growing medium helps save energy for the farming technique and plants. As gravity automatically drains excess liquid,

this technique is specific to use in space. As aeroponics is not a typical method, it is not used as widely. However, it has started to gain more traction with the rise of vertical farming and its efficiency.

Crops suitable for vertical farming

There continues to be increasing interest in vertical farming, especially by entrepreneurs and governments of countries that rely on the importation of food crops. However, because the costs to build and operate a vertical farm are high, only certain types of crops are potentially profitable. The following are few important features that describe suitability of crops in vertical farming systems.

Short production cycle: Considering the high costs of producing crops indoors, crops that can be produced in weeks — not months — lend themselves to indoor farming. The longer it takes to produce a crop, the greater the electricity cost for lighting and operation of the heating, ventilation and air conditioning systems, as well as the greater fixed costs (rent, equipment depreciation, etc.) that must be allocated to that crop.

High harvestable yield: This refers to the portion of the crop that can be harvested and sold. For crops like lettuce, almost the entire plant can be sold and thus, has a very high harvestable yield. In contrast, for a crop like tomato, one can only sell the fruits. The energy used to generate and maintain leaves and stems is essentially "lost" because there is no market for those portions of the plant.

Short stature: Plants that have a compact growing habit are more suitable for vertical farming because the distance between growing layers can be relatively short. Space is used less efficiently with taller crops. Unless the distance between the lights and the plants can be adjusted (which is often not practical), more lighting capacity is needed to reach the plants when they are young. As plants grow closer to the light, the light intensity at the crop canopy increases, but also becomes more variable.

Year-round demand: Profitability usually necessitates continuous operation and thus, there must be sufficient year-round market demand for the crop(s) grown. Producing the same crop year-round is much easier horticulturally, and it allows growing systems to be engineered and optimized specifically for that crop. One can imagine rotating crops seasonally, when the market price for each crop is highest, but this makes automation of the growing and harvesting processes difficult at best.

Limited labour: Vertical farmers often report that labour is one of their largest costs. Thus, crops that can be "sown and grown" with little labour lend themselves to vertical farming.

Automation decreases labour inputs, but it usually requires significant up-front costs to design, purchase and install.

Perishable: One of the virtues of indoor farming is the ability to produce crops close to where they are sold, such as large cities. The shelf life and/or quality of perishable crops can be increased when the period between harvest and reaching the market is short. A short harvest to market time can also reduce shrinkage compared with crops transported long distances.

High value: Because of the greater costs of producing crops indoors, they need to command a relatively high price. We can produce any food crop indoors, but is the price obtained sufficiently high to be profitable?

Food miles

Food miles is the distance that food is transported from the time of its making until it reaches the consumer. Food miles are one factor used when testing the environmental impact of food, such as the carbon footprint of the food as the process of transportation involves burning of fossil fuels which produces greenhouse gases.

Advantages of vertical farming

Despommier's original vision was a world full of skyscrapers with multiple levels cultivating crops throughout the year. In addition to generating more farmland on a single ground-level footprint, this would, according to a review by The Economist (2010), 'slash the transport costs and CO_2 emissions associated with moving food over long distances. It would also reduce the spoilage that inevitably occurs along the way.' In putting forth his pioneering conception, Despommier outlined a number of reasons why vertical farming could be highly attractive to policy makers like: All-year-round crop production, higher yields (by a factor of six or more depending on the crop), avoidance of negative effects of droughts, floods and pests, water recycling, ecosystem restoration, reduction of pathogens, provision of energy to the grid through methane generation from compost, reduction in use of fossil fuels (no tractors, farm machinery, or shipping) and creation of new jobs. The closed environment could conceivably be also suitable for translation to other planetary environments in the context of space exploration. The claimed benefits of vertical farming can be categorized and summarized in terms of economic, environment, social, and political dimensions.

Economic advantages

The economic advantages of vertical farming are numerous and include the prestige of marketing premium CGG food with export-sales potential and a lower cost base due to protection from floods, droughts, and sun damage. There are essentially no requirements for fertilizers, herbicides or pesticides. No soil is needed if hydroponics is used, only nutrients and a water supply. There is no requirement for long-distance transportation due to localized production and no need for farm machinery such as tractors, trucks or harvesters. There are no seasonality issues because continuous crop production occurs all-year round and can be programmed to match demand. An economic benefit may arise from reallocation of large rural farms to energy production from solar and wind sources.

Environmental advantages

The environmental benefits are significant, including providing healthy organic food not contaminated from chemicals. There is greatly reduced use of fossil fuels by avoiding transportation from rural zones to the urban customer base. Burning fossil fuels can be minimized by employing solar panels, roof-top wind turbines, and storage batteries. This will lead to a reduction in ecosystem-carbon levels. Fresh water is augmented by evaporation of black and gray water to conserve water resources. There is also the potential to rejuvenate the national ecosystem so that rural land is reclaimed for vegetation. Most importantly, vertical farming supports environmental sustainability.

Social advantages

Vertical farming will provide new jobs in engineering, biochemistry, biotechnology, construction, maintenance and in research and development opportunities for improving the technology. Enhanced productivity can lead to lower food and energy costs and improve discretionary incomes. The oversupply of high-rise apartments and disused warehouses in capital cities can be reduced by using empty buildings for multi-storey farms close to the consumer, rejuvenating neglected neighborhoods. The model may help to address isolation in remote rural communities by re-skilling workers in technology for vertical farms in local towns and cities.

Political advantages

A key political advantage of vertical farms is that climate-change commitments are more easily satisfied and the technology supports adaptation and mitigation. The closed-system approach supports biosecurity because of greater protection from invasive pest species. A distributed network of vertical farms has lower blackout risks and there is also

reduced dependence on a few large power stations that are vulnerable to earthquakes or terrorist attacks.

Challenges to vertical farming

There have been critics of the original vision of vertical farming as described by Despommier (2010). For instance, Cox claimed that there are a number of problems including the limited range of crops suitable for this business model (originally mostly vegetables such as lettuce, strawberries, and tomatoes), together with the small proportion of the population that could be serviced and the expensive energy requirements. Furthermore, he contends that only the plants on the top level would benefit from solar radiation in a greenhouse environment and energy supplied by photovoltaics is limited because plants cannot be stacked in vertical arrays. The arguments advanced by Cox have become less relevant due to continuing advances in technology. For example, solar panels are now more efficient for energy generation and light exposure is more cost-effective due to the advent of new cheap and energy efficient LED lighting. Additional sun exposure is possible using rotatable stacked arrays of plants inside a single high-rise enclosure. The cost of storage batteries is decreasing rapidly by analogy with Moore's Law in electronics. The new LED sources have potential for greatly increased yield in greenhouse settings due to matching spectral characteristics with plant type and physiology. The challenges to vertical farming may be summarized as follows. Start-up costs can be high if land is purchased in central business districts. The number of crops grown is not as great as for rural farming. Production volumes are also not as large as broadacre farming and scaling-up may add cost and complexity. More specific challenges are the need to manage disruption to the rural sector, to raise investment capital, and to train a skilled workforce.

Key performance indicators

Key performance indicators (KPIs) are metrics that can be used to support the evaluation of vertical farming. The KPIs may be quantifiable or qualitative assessments based on modelling, analysis, literature review, and expert opinion. The factors making up the KPIs are itemized in Table 1. The tabulated values are discussed in greater detail in the following subsections. Note that each KPI identified as satisfied may still be improved in future. An advantage of the KPI table is that it highlights and ranks issues of importance. This methodology also provides the foundations for further analysis if there is a quantitative dimension to the performance indicator.

Research studies

Table 1: Study on attitude of urban people towards vertical farming

Attitude/behavioral intent statement	Agree	Neutral	Disagree
To me indoor farming sounds like a good idea	62	18	20
I would like to try fruits and vegetables grown in indoor farms	**66**	20	13
I would be willing to pay a premium for fruits and vegetables grown in indoor farms	22	18	**60**
It worries me that growing fruits and vegetables indoors will add to the burden of climate change	43	21	36
It will be safe to eat fruits and vegetables grown in indoor farms	**76**	16	8

Source: Sara *et al.,* 2021

Discussion:

Sara and her co-workers carried out a survey on behaviour of people towards vertical farming, wherein 76 % of the people believe produce from VF is safe to eat and 66 % are willing to try fruits and vegetables grown in indoor farms.

But 60 % of the respondents are not willing to pay premium amounts. It is a challenge to researchers to develop system with low cost of production for its sustainability.

Table 2: Economic comparison between hydroponic and soil grown system

System	Fixed cost (Rs.)	Variable cost (Rs.)	Total (Rs.)	Remark
Hydroponic system	44,255	3,507	47,762	120% more compared to geoponics
Geoponics	20,149	1,492	21,641	

Source: Chenin and Stanley, 2015

Compared to traditional systems, hydroponic system is requiring greater cost of production and it is around 120% higher than traditional system.

Table 3: Salinity limits (EC) of irrigation water and root environment for different crops grown in VF

Crop	EC in root environment (ds/m)	EC of irrigation water (ds/m)
Tomato	3.8	3.2
Cucumber	3.5	2.8
Lettuce	2.5	2.3
Sweet pepper	3.5	2.8
Eggplant	3.5	3.0
Strawberry	2.0	1.5

Source: Farnoosh *et al*., 2016

Table 4: Components of IVFS and its economics in Tripura, India

Particulars	**Components of Integrated Farming System**			
Breed	**Goat (Black Bengal)**	**Poultry (Grower) Colour Broiler**	**Poultry (Layer) Grampriya**	**Rabbit (Soviet Chinchilla)**
Space (ft^2)	14 x 10	14 x 10	50 x 2	12 x 3
Population	8	200	100	12
Duration (days)	365	90	365	365
Number of batches	1	4	1	1
Weight gain (kg/batch)	24	1.6	2.2	2.9
Production cost (Rs./batch)	19200	64000	36000	8600
Net profit (Rs./year)	37200	89600	39800	17800
B:C ratio	**3.12**	**2.4**	**2.10**	**3.06**

Source: Singh and Das (2018)

Discussion: The main objectives of system were:

(i) Subsidiary source of income,

(ii) Year-round food security and nutritional benefits, and

(iii) Maximum utilization of available family labour

- Designed with effective area of 630 sq. feet
- Total annual expendi. 1.3 lakh, Net profit- 1.85 lakh
- Construction cost- 1.5 lakh.

Conclusion:

Vertical farming has enough potential to supply fresh fruits and vegetables in urban areas and can reduce considerable amount of adverse effects of food miles on environment. Care must be taken in selecting crops since vertical structures are not suitable to grow all crops like open field and also certain financial aspects are to be considered. Vertical farming with its inherent political, ecological and environmental benefits can be considered at priority for expanding its spread.

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