

**Shri Sarda Education Society's
(A Linguistic Minority Educational Institute)**



**Smt Radhabai Sarda Arts, Commerce & Science College
Anjangaon Surji, Dist - Amravati, Maharashtra
NAACAccredited 'A' with CGPA 3.21**

Department of Botany

FIELD PROJECT/ SURVEY REPORT

**B.Sc.III SEM-VI
2023-2024**

Department of Botany

FIELD PROJECT / SURVEY REPORT

Department of conducted field project and Survey related to Banana Ripening Center in Anjangaon Tahsil- Natural & Artificial Ripening, Case study on Banana Sapling preparation & Hardening by tissue culture (Nearest Tissue culture Laboratory) Transgenic food Crops Cultivated in Anjangaon Region (Krushi Kendra Survey) etc. prescribed by S G B Amravati University Amravati for B.Sc. III SEM-VI (Molecular Biology, Biotechnology and Genetic Engineering) students. Fifty one students participated in field project and survey.

Participated students in Filed project and Survey:

SN	TITLE OF PROJECT/ SURVEY	CLASS	STUDENT NO
1	Banana Ripening Center in Anjangaon Tahsil- Natural & Artificial Ripening	B.Sc. III SEM-V	51
2	Case study on Banana Sapling preparation & Hardening by tissue culture (Nearest Tissue culture Laboratory)		
3			
	<i>Other topic as per list attached</i>		51



Dr Mangesh Dagawal
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Principal
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Commerce & Science College
Anjangaon Surji

List of Students and Projec

SMT RADHABAI SARDA ARTS, COMMERCE AND SCIENCE COLLEGE, ANJANGAON SURJI DIST AMRAVATI

DEPARTMENT OF BOTANY

INTERNAL ASSESSMENT 2023-2024

Project Assignment - Field Project/ Project -Summer-2024

B. Sc-III SEM- VI

SN	NAME OF STUDENT	Project Assignment/ Field Project / Field Survey - TOPIC –	NAME OF GUIDE	SUBMITTED ON DATE& SIGNATURE
1	Om Pramod Khode	Banana Ripening Center in Anjangaon Tahsil- Natural & Artificial Ripening	Dr Mangesh Dagawal	
2	Priti Dipak Bodkhe			
3	Natique Kamran Zameeruddin			
4	Prajakta Babarao Bansod			
5	Pratik Shridhar Kale			
6	Tejas Dilip Bhople			
7	Asmita Chandrabhan Wanere			
8	Archana Chandrabhan Wanere			
9	Gayatri Rajesh Deshkar			
10	Sejal Sudhirrao Khalokar			
11	Yashasvi Satishrao More	Case study on Banana Sapling preparation & Hardening by tissue culture (Nearest Tissue culture Laboratory)	Shri Ramchandra Kulkarni	
12	Shital Gulabrao Hire			
13	Mohini Ramdas Kedar			

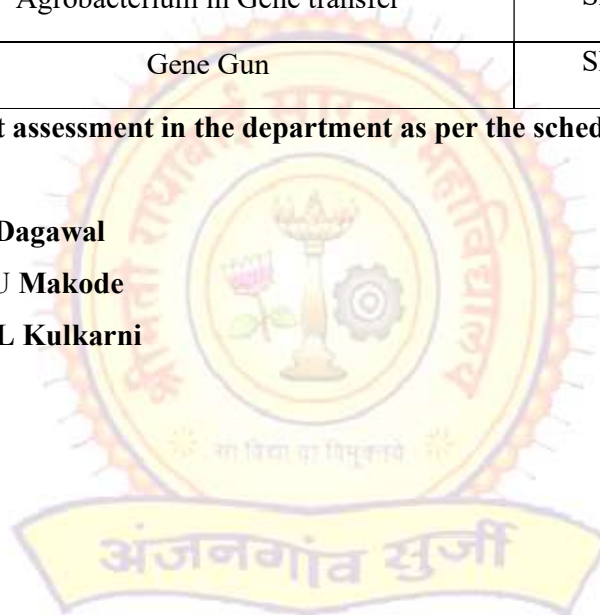
14	Nikita Nandu Raibole			
15	Mahvish Anam Mohd Nazim			
16	Prathamesh Surendra Nimkar			
17	Saniya Firdous Shaikh Rauf			
18	Kashish Sharad Lahane			
19	Javeriya Tabassum Aqueel Ahmed			
20	Sanchit Vinod Kukde			
21	Rohit Ramdas Jadhav	Transgenic food Crops Cultivated in Anjangaon Region (Krushi Kendra Survey)	Dr Mangesh Dagawal	
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29	Priya Wasudevrao Badan			
30	Krushnakant Mukundrao Kadu			

31	Adesh Bapurao Misar	Antisense RNA Technology	Shri Ramchandra Kulkarni	
32	Nilesh Vinayakrao Dhumale	DNA Packaging	Shri Ramchandra Kulkarni	
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Name & Signature of Teacher Incharge **Dr M J Dagawal**
Shri K U Makode
Shri R L Kulkarni



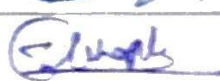
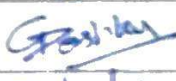
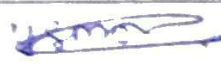
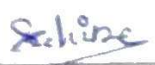

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DEPARTMENT OF BOTANY

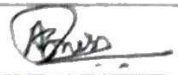
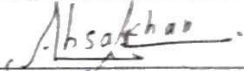
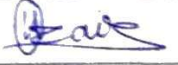
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B. Sc-III SEM- VI

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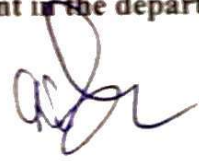
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**SMT. RADHABAI SARDA ARTS,
COMMERCE AND SCIENCE COLLEGE,
ANJANGAON SURJI.**

DEPARTMENT OF BOTANY

2023-24

B.SC-III SEM-VI

SUMMER 2024

FIELD PROJECT

**TOPIC- BANANA RIPENING CENTER IN
ANJANGAON SURJI TAHSIL- NATURAL
AND ARTIFICIAL RIPENING**

GUIDED BY – DR. MANGESH DAGAWAL

CERTIFICATE

**Name of college – SMT. RADHABAI SARDA
ARTS, COMMERCE AND
SCIENCE COLLEGE,
ANJANGAON SURJI.**

Name of Department – Botany

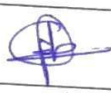
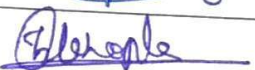
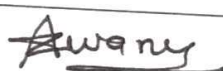
**This is to certify that Om Pramod Khode,
student of B.Sc part-III (Semester-VI) has
successfully completed their given project from
02/03/2024 to 06/04/2024, during Academic year
2023-2024.**

Guided by-

Dr. Mangesh Dagawal



**Head of
Department**

SR. NO.	NAME OF STUDENT	SIGNATURE
1	OM PRAMOD KHODE	
2	PRITI DIPAK BODKHE	
3	NATIQUE KAMRAM ZAMEERUDDIN	
4	PRAJAKTA BABARAO BANSOD	
5	PRATIK SHRIDHAR KALE	
6	TEJAS DILIP BHOPLE	
7	ASMITA CHANDRABHAN WANARE	
8	ARCHANA CHANDRABHAN WANARE	
9	GAYATRI RAJESH DESHKAR	
10	SEJAL SUDHIRRO KHALOKAR	
		


 Head
 Department of Botany
 Smt. Radhabai Sarda Arts,
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INTRODUCTION:

A banana is an edible climacteric fruit produced by several kinds of large herbaceous flowering plants in the genus *Musa*. The fruit is inconsistent in size, colour, and firmness but is generally elongated and bowed, with flexible flesh rich in starch covered with a rind, which may be red, yellow, green, brown, or purple while ripe. Banana has to ripen completely so that it has its fullest taste and nutrients. Post ripened harvesting of banana leads to lesser shelf life and it is not suitable for long transportation hence bananas are harvested when they are $\frac{3}{4}$ matured. These harvested pre-ripened bananas are transported and made to ripe at the place of retail markets using artificial ripening agents so that they attain uniform ripening and more appealing physical and sensory character. The major problem that plays here is the use of banned chemicals and harmful methods for artificial ripening thus causing severe health hazards.

Most of the ripening agents are poisonous and their utilization can cause severe health problems, such as heart disease, lung failure, skin disease and kidney failure^{1,2}. Artificial ripening may accelerate the ripening process but it affects the nutritional quality sensory and safety of fruits³. The common nutrient contents and vitamin C content are found high in naturally ripened banana when compared to the artificial ripened one but the shelf life is straight opposite in nature.

Based on the characteristics of artificial ripened used in artificial ripening can be classified as:

Natural artificial ripening:

The ripening process is carried out by means of natural ripening agents and methods (without the aid of chemical substance). Example: Ripening by fumes, increasing the ripening rate by placing in a closed container, placing fruits like apple, avocado etc. along with unripe bananas promotes ripening to a greater extent.

Synthetic artificial ripening:

Various chemical compounds are used in greater extent to induce and promote the ripening. The ripening method usually involves chemicals either in the form of solid (calcium carbide), liquid (ethrel) and gaseous (ethylene) in nature.

Natural methods:

The simple method practiced in the household to trigger ripening is to keep the unripe fruit inside an air tight drum containing rice or wheat. The fruits stored in such a way ripen quickly because of the accumulation of ethylene gas and self heating nature of grains. Rice and wheat grains differ in their seed dormancy which affects the seed respiration rate. Respiration of rice or wheat grains produces carbon dioxide and this, in turn, induces ethylene biosynthesis in a number of fruits. Since respiration is more in rice than wheat grains unripe fruits kept in rice ripen faster than those kept in wheat grains. Unripe fruits spread as layers over paddy husk or wheat straw for a week to ripen is another alternative.

In an experiment where packaging materials such as plastic bag, poly sheet, wooden box, open air as control and plant ethylene sources such as avocado, mango, tomato were used and considered as two factors. One packaging material contains one plant ethylene resource at a time where by, each packaging material gets the equal weight of the three ethylene sources in separately. Hence there were 12 treatments with three replications accounting for 36 experimental units. Samples of unripe bananas were placed in each unit. The results showed that banana placed with avocado ripened first in 6.4 days followed by tomato in 6.5 days and mango in 7.1 days. In relation with containers bananas placed in wooden box ripened first and bananas placed in poly sheet took the longest duration for ripening. Based on this experiment avocado in combination with the wooden box can be used to get the shortest ripening period without any undesirable effect on the quality of the fruit.

Placing bananas with other fruits:

Apple:

The banana ripening which is induced by using apple, provide ripened bananas with higher nutritional and organoleptic qualities.

Avocado, Mango and Tomato:

When these three are individually used as a source of ethylene ripening with avocado fruits showed the shortest ripening period without any adverse effect on quality, followed by mango and tomato.

Temperature:

Increase in storage temperature between 14°C and 30°C promotes the rate of ripening and softening of fruits⁷ while higher temperature also leads to damage in ripening⁸. At lower temperature ripening permanently stalls, banana turns grey as cell wall break downs. The skin of banana quickly blackens at 4°C although the fruit inside remains unaffected. At 13°C bananas are stopped producing their natural ripening agent ethylene and in this way they can be transported to very long distance for 3-4 week duration and on arrival they are held at 17°C, in few days bananas will ripen and will be ready for sale in the market.

Chemical methods:

Calcium carbide (CaC₂):

Calcium carbide is a highly hazardous chemical for living organisms, hence it is banned. Since it is low in cost, it is abundantly used in the artificial ripening process. Calcium carbide when it comes in contact with moisture it produces acetylene which has similar effects as ethylene but calcium carbide has traces of arsenic and phosphorous which are highly toxic and carcinogen that makes this chemical unfit to use for ripening.



It has various short and long term health effects. Contact causes irritation or burns in skin or eye causing permanent eye damage. It also causes irritation in

mouth nose and throat causing coughs and wheezing. Higher exposure may cause pulmonary edema. Because of its alkaline nature, calcium carbide causes stomach disorder by irritating the mucosal tissues of the abdomen¹². It reduces the oxygen supply to the brain and affects the nervous system¹⁴. It is more vulnerable to pregnant women as the chemical residue cause miscarriage. Calcium carbide can induce ripening within 24 h and it is the cheap and most used technique in developing countries^{10,11}. The available calcium carbide in the market is grey or brown in colour and contains 80-85% calcium carbide.

The qualitative test for the fruits ripened by using calcium carbide have uniformly coloured texture but not uniformly coloured in naturally ripened fruit, similarly aroma is mildly good by carbide whereas good by naturally, taste is in core sour and mildly pleasant in artificial, but sweet and pleasant by natural method. The shelf life is shorter in artificial method whereas longer in natural method. The firmness is fair in both the ripened methods.

Ethylene (C₂H₄):

Safe and worldwide accepted way of inducing ripening is the usage of ethylene, a natural plant hormone under controlled concentration, temperature and relative humidity condition¹⁷. The concentration of ethylene used for ripening is 100-150ppm and its exposure time is 24 h. During ripening the temperature is maintained at 15-18°C and the storage temperature is 13-14°C. The relative humidity is maintained at 90-95%. The Food Safety and Standards Regulation, 2011 has permitted the use of ethylene gas at a concentration is up to 100ppm. This is non-toxic for human consumption but it is expensive.

Methyl jasmonate (C₁₃H₂₀O₃):

Jasmonic acid and methyl jasmonate greatly promote the ripening process in non-climacteric fruits. Studies have shown that the addition of methyl jasmonate increases the gene expressions that are much involved in the ripening process. This method is also stated as non-toxic and highly expensive.

Ethephon (C₂H₆ClO₃P):

Ethephon (ethrel) in the presence of moisture and neutral pH is decomposed into ethylene gas, biphosphate ion and chloride ions¹³. These chemicals have adverse effects on human health. It gives more acceptable colour and greater shelf life than fruits ripened with calcium carbide¹¹. It produces toxicity including salivation, lacrimation, urination and defecation¹⁴. Ethephon treated bananas have more nutritious factors when compared with calcium carbide treated bananas¹⁸. 500ppm of ethrel solution is optimum for inducing uniform ripening of 75-80% mature bananas in 6 days at $20\pm 1^{\circ}\text{C}$. At least this can be used as a replacement for calcium carbide since it is less toxic.

Ethrel with Sodium hydroxide:

Another simple and harmless technique is 10ml of ethrel and 2g of sodium hydroxide pellets are mixed in five litres of water taken in wide mouth vessel. This vessel is placed in a ripening chamber near the fruits and the room is sealed air tight. Ripening of fruits takes place in about 12 to 24 h.

Ethylene glycol ($\text{C}_2\text{H}_5\text{O}_2$):

Chemically synthesised ethylene glycol is also used in the artificial ripening process. The main impurities are diethylene glycol, triethylene glycol, methanol and aldehydic oxidation products. It also possess a harmful effect and may cause kidney failure.

Calcium chloride (CaCl_2):

Calcium chloride soaked and ripened bananas show 60% decreased vitamin C²². Hence this process is not recommended.

Smoke treatment:

Smokes are created using straw, leaves and cow dung in a closed chamber with heaps of banana placed inside. The duration is about 18-24 h in summer and 48 h in winter. Later it is placed for development of colour in a well-ventilated room. In this method, ripening takes place in 3 days. Fruits ripened by this

method are less preferred because of cuts, bruises, microbial infection and poor appearance²³. The same thing may also be carried out in the pots as small scale. Fruits which are exposed to 30 h of heat produced by burning dry straw are ripened in 3.4 to 3.8 days and had a shelf life of about 8 days while untreated required 10 days to ripe fully and had a shelf life of about 11 days²⁴.

When we compare the ripening process in smoke using straw method the gradual difference was observed in three days with the control one. In case of first day the texture, firmness and the taste of the control will be green, very hard and sour whereas treated will be green with yellow spots, hard and sour in core and the second day control will be yellowish green, hard and sour and the treated will be yellowish green, soft and sweet and the third day will be yellow, mild soft and sweet but sour in core and the treated will be yellow, completely soft and sweetish taste respectively.

Smoke generated by burning kerosene stove inside air tight chamber is also used for inducing ripening but this treatment is toxic as the smoke generated by kerosene contains carbon monoxide and thus lays harmful. It also contains impurities like sulphur which cause pulmonary injuries and may be fatal²⁵.

From the study it revealed that bananas that are fumed and opened after three days are very soft and sweet to eat when compared to a standard which is kept normally at room atmosphere. Since here the fumes are induced by straw and dried leaves there is no traces of hazardous chemical compounds and it is completely safe. This method is completely non-toxic and can be opted as an alternate instead of using hazardous artificial ripening agents. From the reviewed papers it is evident that natural methods of ripening are completely non-toxic. Ethylene, methyl jasmonate and the combination of ethrel and sodium hydroxide are chemicals which have no harm when used in artificial ripening because it does not come in direct contact with the fruits. Other chemicals such as ethephon, calcium chloride, ethylene glycol and most importantly calcium carbide do have high dangerous levels of toxicity. In natural methods avocado placed unripe bananas ripen at the earliest. Likewise, unripe banana stored in the wooden box ripens quickly when compared to storage materials like a poly sheet or poly bag. Unripe bananas which are placed in rice ripen faster than the ones which are placed in wheat grains.

Ripening of Bananas with Ethylene:

- Bananas are received at the ripening plant at color stage 1 (refer color chart above).
- The product is pre-cooled to the pulp temperature of 18°C.
- After stabilization of temperature, ethylene is introduced at a concentration of 100 – 150 ppm.
- Most convenient and safe method of introducing ethylene is through ethylene generators.
- Cylinders containing 5% mixture of ethylene gas with nitrogen may also be used.
- After 24 hours, the chambers are vented. During this period the fruit starts producing ethylene by itself at the rate of 2 - 4 $\mu\text{l} / \text{kg} \cdot \text{hr}$.
- Ripening associated changes include an increase in rate of respiration from 20 to 60 - 80 ml $\text{CO}_2 / \text{kg} \cdot \text{hr}$ and a similar 3 to 4 fold increase in heat production.
- The product breathes in oxygen and gives off carbon dioxide.
- Excess ethylene and carbon dioxide must be removed for faster and uniform ripening.
- The refrigeration system must be capable of removing the heat of respiration and maintain the temperature and relative humidity at desired levels.
- The venting system must be capable of removing excess carbon dioxide in order to maintain concentration level of below 10,000 ppm.

Ripening Cycle:

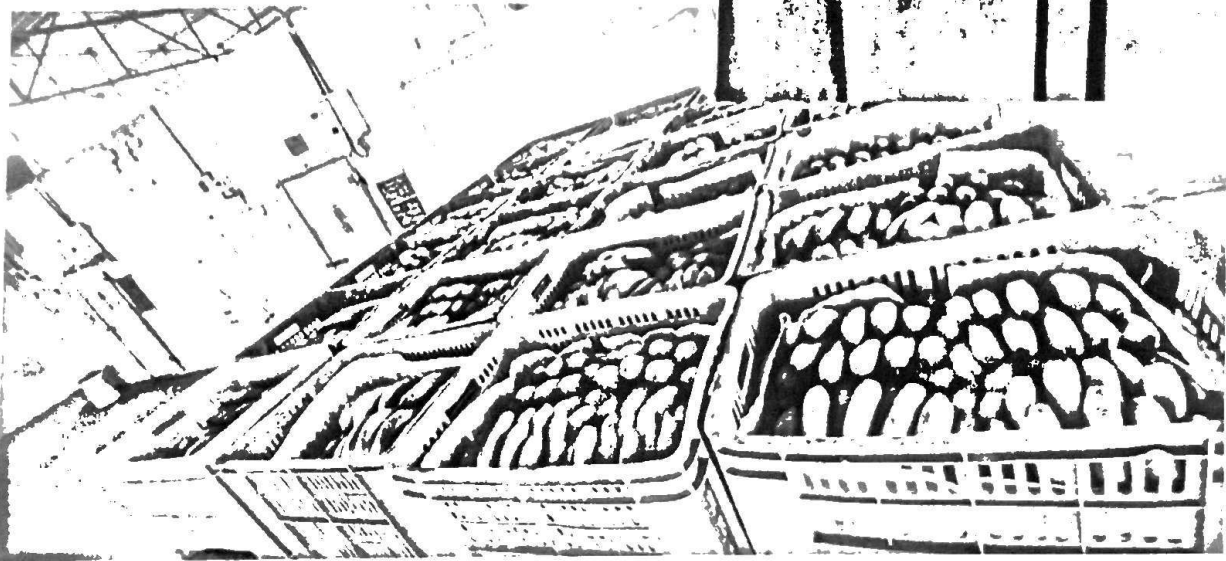
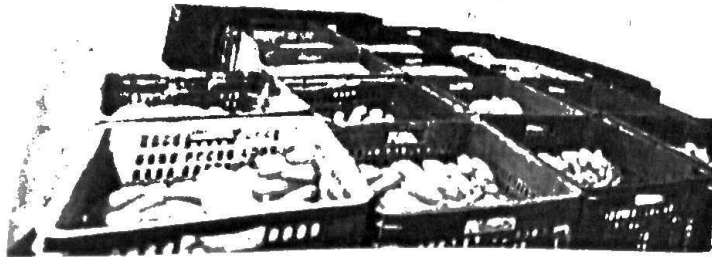
- Bananas are ripened to color stage 3 or 4 depending on mode of retailing for dispatch to market.
- Depending on cultivar and market requirements, ripening cycle may be spread from 4 to 8 days.

PHYSICOCHEMICAL CHANGES:

When considering the physicochemical characters there is a decrease in weight and dry matter content and increase in specific gravity with increase in storage time. The total soluble solids increases and titrable acidity generally decreases when being treated with chemicals for ripening. Highest amount of vitamin C content was found with naturally ripened banana. The physiological and biochemical changes that occur during ripening of banana with or without the aid of artificial ripening agents that are studied from various research papers are tabulated and summarized below in Table 2. It is always evident that the bananas which ripen naturally has the best biochemical composition and nutrients that has highest beneficial to humans when consumed rather than artificially ripened bananas.

Photos of Banana Ripening Centre-

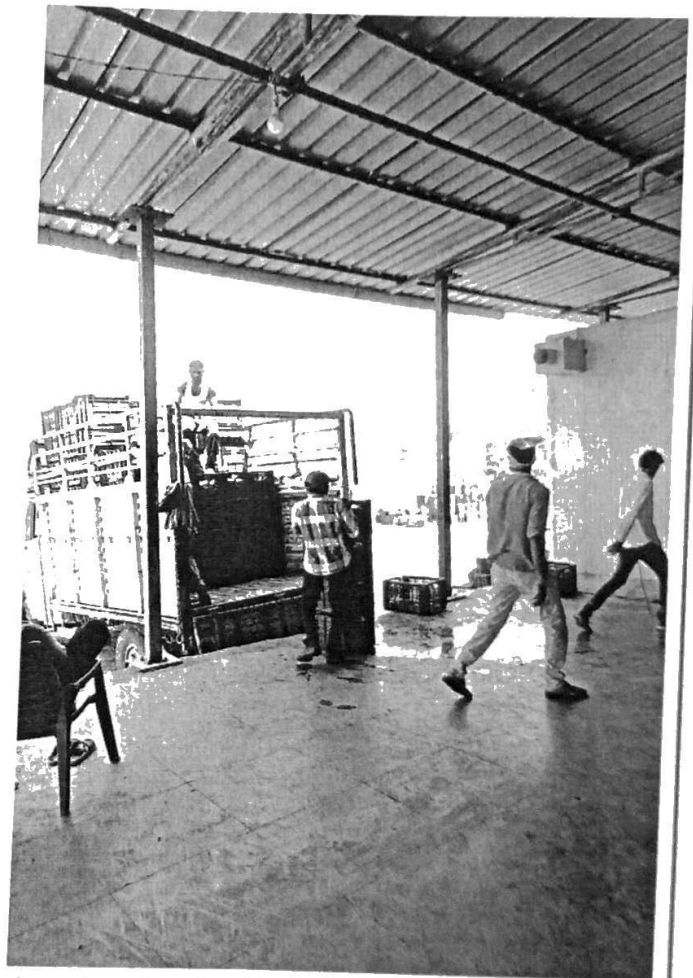






CONCLUSION:

It is evident and already proved by many researchers that usage of chemical agents such as calcium carbide, ethephon, ethylene glycol, calcium chloride and inducing ripening by fumes from kerosene is highly hazardous and may be fatal if consumed. It is always best to



use naturally induced methods of artificial ripening by placing bananas with avocado, apple etc or by using fumes from dried leaves or straws. In the case of using chemical agent's ethylene, methyl jasmonate and ethrel with sodium hydroxide combination can be used because of non-toxic. But practically vendors are not using this because it is too expensive. Alternatively, ethephon at a concentration of 1ppm-50ppm can be used; it is non-hazardous if only maintained at this particular concentration. When considering the physicochemical characters there is a decrease in weight and dry matter content and increase in specific gravity with increase in storage time. The total soluble solids increases and titrable acidity generally decreases when being treated with chemicals for ripening. Highest amount of vitamin C content was found with naturally ripened banana. Hence it is concluded that the order of beneficiary of faster ripening in relation to packing materials is wooden box> poly bags> poly sheet and in relation to chemicals used the order is ethylene> ethrel with sodium hydroxide>methyl jasmonate> ethephon> calcium chloride> ethylene glycol> calcium carbide. It is always best to artificially ripe bananas by the naturally induced method or using chemical methods that are non-toxic if not it can be left as such to ripen without using ripeners.

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Smt. Radhabai Sarda Arts, Commerce and Science College, Anjangaon Surji

Department of botany

Session 2023-24

Class- B. Sc III Semester VI (PCM)

**A project report
On**

**TRANSGENIC PLANTS CULTIVATED
in Anjangaon Surji, Dist. Amravati**

Submitted by

KRUSHNAKANT M KADU AND GROUP

Guided by

Dr.MANGESH DAGWAL SIR

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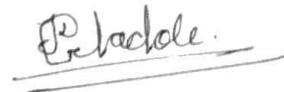
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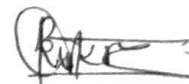
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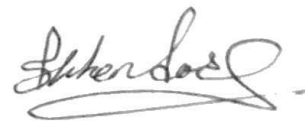
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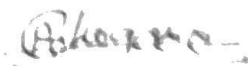
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Name of Department – Botany

**This is to certify that Prajakta Babarao Bansod,
student of B.Sc part-III (Semester-VI) has
successfully completed their given project from
02/03/2024 to 06/04/2024, during Academic year
2023-2024.**

Guided by-

Dr. Mangesh Dagawal


**Head of
Department**



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😊 INTRODUCTION

😊 Bt COTTON

😊 MAJOR PESTS OF COTTON

😊 MODE OF ACTION OF Bt GENE

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😊 CONCLUSION

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WHAT IS Bt COTTON?

- Genetically modified variety of cotton that produces an **insecticide** whose gene has been derived from a soil bacterium called *Bacillus thuringiensis* (**Bt**).
- Three types of toxins.
- A total of **229** cry toxins (cry1Aa to Cry72Aa), cyt toxins (cyt 11Aa to cyt3Aa) and 102 vip toxins(vip1Aa1 to vip4Aa1) have been discovered.

Bacillus thuringiensis

- Gram positive soil bacterium.
- Commonly used as a biological pesticide.
- It was discovered in 1901 by **Ishiwatori Shigetane** in silkworm.
- Many Bt strains produce crystal proteins called **δ -endotoxins** during sporulation, that have insecticidal action. This has led to their use as insecticides.

MODE OF ACTION OF Bt GENE

- *Bacillus thuringiensis* serves as an important reservoir of **cry** toxins and cry genes for production of biological insecticides and insect-resistant genetically modified crops.
- The Bt gene was isolated and transferred from bacteria to American cotton to produce **Bt cotton**.
- This cotton variety produces Bt toxin **Cry1Ac** which is particularly specific to American **Bollworm**.

DEVELOPMENT OF Bt COTTON

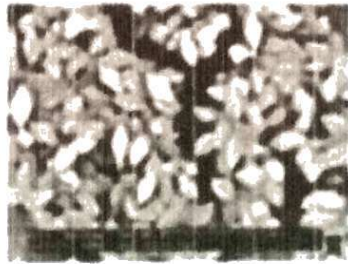
- Main steps for developing transgenic crops :

1. Identification of effective gene(s)
2. Gene transfer
3. Regeneration from protoplast/callus/tissue
4. Gene expression to the desired level
5. Back cross to produce varieties
6. Field test
7. Approval for commercialization

MONSANTO	Cry 1Ac and Cry 1Ac+Cry 2Ab
CHINESE ACADEMY OF AGRICULTURAL SCIENCES THROUGH NATH SEEDS	Cry 1Ac
METAHELIX	Modified Cry 1Ac
JK SEED	Cry 1Ac

CONCLUSION

- Bt Cotton is produced by inserting a synthetic version of a gene from the naturally occurring soil bacterium *Bacillus thuringiensis*, into cotton.
- The primary reason this is done is to induce the plant to produce its own Bt toxin to destroy the **bollworm** (*Helicoverpa sp*), a major cotton pest.
- The gene causes the production of δ -endotoxin in all parts of the cotton plant throughout its entire life span. When the bollworm ingests any part of the plant, the Bt cotton toxin pierces its small intestine and kills the insect.





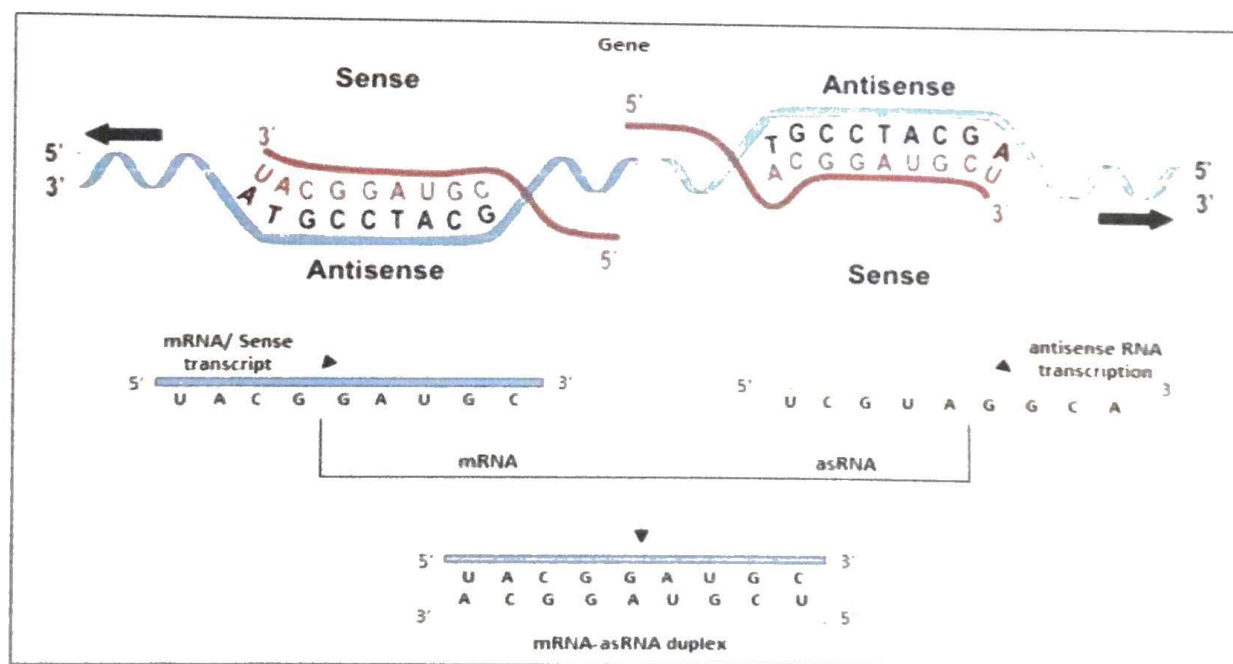
**SMT RADHABAI SARDA ARTS, COMMERCE & SCIENCE COLLEGE,
ANJANGAON SURJI**

DEPARTMENT OF SCIENCE

SUBJECT :- BOTANY

Class :- BS.c III (Final Year) Sem VIth

TOPIC NAME :- Antisense RNA Tecnology



GUIDED BY :-

DR. MANGESH DAGAWAL

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CERTIFICATE

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Abstract

Antisense RNA (asRNA) technology is a method used to silence genes and inhibit their expression. Gene function relies on expression, which follows the central dogma of molecular biology. The use of asRNA can regulate gene expression by targeting specific mRNAs, which can result in changes in phenotype, disease resistance, and other traits associated with protein expression profiles. This technology uses short, single-stranded oligonucleotide strands that are complementary to the targeted mRNA. Manipulating and regulating protein expression during its translation can either knock out or knock down the expression of a gene of interest. Therefore, functional genomics can benefit from this technology since it allows for the regulation of protein expression. In this review, we discuss the concept, and applications of asRNA technology which include delaying ripening, prolonging shelf life, biofortification, and increasing biotic and abiotic resistance among others in crop improvement and sustainable agriculture.

Introduction

The world's population is rapidly increasing, and there is a need to improve food crop production and productivity to meet the growing demand (Godfray et al. 2010; Saurabh et al. 2014; Rajam 2020; Rajput et al. 2021). Traditional plant breeding has been used since the domestication of crops to enhance productivity, environmental adaptation, and quality (Miflin 2000; Animasaun et al. 2020). Today, both conventional and non-conventional breeding methods, including molecular breeding, crop gene editing, mutation breeding, tissue culture, hybridization, antisense technology, and crop genetic transformation, are utilized to develop improved crop varieties. However, the non-conventional methods are faster and more precise than conventional methods (Auer and Frederick 2009; Wang et al. 2023). Thus, advances in biotechnology and genetic engineering tools have made crop improvement more convenient and less cumbersome (Lemmon et al. 2018; Tilahun et al. 2021; Che et al. 2022), even in the production of clean energies (Yan et al. 2020).

Since the discovery of small interfering RNA (siRNA), long noncoding RNA (lncRNA), microRNA (miRNA), and PIWI-interacting RNA (piRNA), typically non-coding RNAs (ncRNA), they have generated popular research topics. Antisense RNA (asRNA) which is a form of ncRNA participates in the regulation of the genetic activity of the chromosome structures, transcription and translation as well as the RNA and protein stabilities (Rusk 2015; Xu et al. 2018). The asRNA is a unique form of the small noncoding molecule of the DNA transcript that complements specific mRNA. Antisense RNA technology involves manipulating the RNA molecule to prevent the expression of a specific gene. Though previously thought to be a "junk RNA" (Boland 2017), asRNA possesses immense potential for intercellular gene interaction and can be a veritable tool for gene silencing. In some cases, asRNA, hairpin (HP) as well as hairpin antisense (HPAS) construct was used to improve plant (Nguyen and Shanklin 2008).

Gene expression involves two main steps: transcription, in which the DNA sequence serves as a template to create an mRNA molecule, and translation, in which the mRNA is used as a template to synthesize a

protein (Clancy and Brown 2008; Gupta et al. 2011). The genes in DNA provide the instructions for making proteins, which are essential for the functions of the cell. During translation, the mRNA sequence is translated into a specific sequence of amino acids, which form a polypeptide chain that folds into a functional protein (Barry 2007; Clancy and Brown 2008). The genetic code is based on a triplet code, where each codon consisting of three nucleotides specifies an amino acid. Several enzymes, including polymerase, exonuclease, ligase, etc., work in concert to unwind the DNA double helix and match RNA base pairs with the DNA bases to produce the RNA copy of the DNA. The produced mRNA molecule is immature, heterogeneous nuclear RNA (hnRNA) (Clancy and Brown 2008; Kumar 2010; Lemmon et al. 2018) that is not yet for gene expression. The introns of the pre-mRNA must be spliced out and shielded through a 5' cap and poly-A tail addition to protect the mRNA from degradation. (Gupta et al. 2011). The pre-mRNA is subsequently moved out of the nucleus into the cytoplasm where it is transformed into mRNA in preparation for the next step (translation). The translation occurs in the cytoplasm, the mRNA binds to the ribosomes which are responsible for protein synthesis. The description of the basic workflow of the central dogma is illustrated in Fig. 1.

Gene silencing

Gene silencing is an effective molecular procedure that inhibits the expression of a specific gene, and it serves as a regulatory mechanism for gene expression, determining cell fate and metabolic activities during an organism's lifespan. Gene silencing eliminates the effect of a gene by inhibiting its expression (Dash et al. 2015; El-Sappah et al. 2021; Woodward 2022). The presence of double-stranded RNA (dsRNA) in the cytoplasm is the primary factor that induces gene silencing (Fire et al. 1998; Mu et al. 2018). In plants, gene silencing is critical for protection against abnormal RNA, which can be fatal if it persists in the RNA pool of a cell (Dunoyer et al. 2006). This is achieved through nonsense-mediated mRNA decay (NMD) which could also be used for antisense therapeutic purposes (El-Sappah et al. 2021; Woodward 2022).

Following the central dogma of molecular genetics, gene silencing in plants occurs in two stages: transcriptional gene silencing (TGS) and post-transcriptional gene silencing (PTGS) (Kelly and Aramayo 2007). Transcriptional gene silencing occurs in the nucleus and involves blocking the binding of the transcriptional system to the promoter region, thus inhibiting transcription. PTGS, on the other hand, occurs in the cytoplasm and targets and degrades or blocks mRNA transcripts of specific genes (El-Sappah et al. 2021). Examples of TGS techniques include RNA-directed-DNA methylation (RdDM), paramutation, transposon silencing, genomic imprinting, transgene silencing, and position effect whereas Examples of PTGS techniques include RNA interference (RNAi), antisense RNA (asRNA), clustered regularly interspaced short palindromic repeats (CRISPR/Cas9), and non-coding RNA (NMD) (Wakimoto 1998; Vaucheret et al. 2001; Zebec et al. 2016). Small molecular RNAs such as the asRNA could induce the silencing of a sequence-specific gene by inhibiting the transcription or translation process. Consequently, post-transcriptional gene silencing (PTGS) has been reported in various organisms (Matzke et al. 2001), and in some cases, the phenomenal suppression of the expressions for both and the homologous endogenous genes was observed (Campbell 2005).

Antisense RNA

Antisense RNA technology involves modifying RNA molecules to create a complimentary copy of the target messenger RNA (mRNA) in a reverse orientation. This complimentary copy, also called antisense RNA, can bind to the target mRNA, and regulate or inhibit the expression of the corresponding protein (Gupta et al. 2011; Tiwari et al. 2014). Antisense RNA exists in the form of antisense oligonucleotides (ASO/AS-ONs), single-stranded deoxyribonucleotides designed to complement the target mRNA (see Fig. 2). These AS-ONs are usually between 15 and 30 nucleotides in length, and they can be used to effectively regulate gene expression (Crooke 2004; Xu et al. 2018). Zamecnik and Stephenson (1978) were the first scientists to propose the use of small oligonucleotides (ODNs) for targeted gene expression inhibition. Their studies demonstrated that a 13-ODN that complemented the terminally repeated sequences in Rous sarcoma virus (RSV) long terminal repeat (LTR) could prevent Rous sarcoma virus translation in a cell-free system, as well as viral replication in cultured cells in vitro. As time passed, researchers began to fully grasp the potential of antisense-mediated gene regulation.

In the early 1980s, the automation of ODN synthesis made it easy to obtain ODNs of any sequence and evaluate their effectiveness in blocking gene expression via antisense base pairing (Scherer and Rossi 2003; Mo et al. 2012). Antisense oligonucleotides are designed to bind with targeted mRNA via Watson-Crick (1953) base pairing, which involves C-G and A-T. The "sense strand" refers to the original sequence of the DNA or RNA molecule, while the "antisense strand" refers to the complementary sequence of the DNA or RNA molecules (Gupta et al., 2011). The process of hybridizing antisense nucleic acid sequences with a particular mRNA sequence can disrupt the normal processing of genetic information within a cell through various mechanisms. This disruption is either a "knockdown" or a "knockout," depending on whether the information is partially, or completely removed. Thus, the gene function can be determined (Walder and Walder 1988; Mol et al. 1990).

Antisense technology is one of the novel and convenient techniques that crop breeders use to develop and improve crop varieties. Among the tools available to plant biotechnologists, the antisense RNA strategy is especially potent. This approach allows for the targeted reduction of specific gene expressions, resulting in the production of plants with modified traits. It is therefore likely to inhibit characteristics that are deleterious, creating a novel plant mutant for contemporary applications in plant breeding (Bird and Ray 1991; Tilahun et al. 2021). In addition, antisense technologies are employed in functional genomics (Mo et al. 2012).

Synthesis of antisense oligonucleotides

Solid-phase and enzymatic methods are used to synthesize antisense oligonucleotides (Zaramella et al. 2004). The enzymatic method involves the use of DNA-dependent RNA polymerase enzymes such as *sp6*, T7 and T3 enzymes. These enzymes catalyze the synthesis of RNA from the 5'-3' direction, using either single-stranded or double-stranded DNA as templates. These occur downstream of the DNA template (Ji et al. 2017). In a clearer term, during transcription, the template or non-coding or antisense strand of the

2013), and the Argonaute proteins- AGOs (Riley et al. 2012). Essentially, the concept involves introducing a short RNA or DNA molecule into a cell that is complementary to the mRNA produced by a specific gene. By taking advantage of the unique specificity of complementary base pairing as explained by Watson and Crick in 1953, the oligonucleotide can specifically bind to the target mRNA. Consequently, the introduction of an antisense oligonucleotide (ASO) complementary to the mRNA will result in the specific inhibition of protein synthesis by the targeted gene through either cleavage or blockage. Usually, antisense RNA technology involves the overture of a molecular construct with a target gene replicated downstream to a promoter in an inverse orientation promoting the synthesis of antisense RNA complementary to the targeted gene. The duplex formed by the gene and the asRNA complement is subsequently cleaved by a Dicer into short RNA (siRNA) fragments which are integrated into a multi-protein molecule of RNA Induced Silencing Complex (RISC). When RISC is activated, one strand of the siRNA usually refers to as the "passenger" becomes degenerated while the other strand (guide) sticks with the complex (Matzke and Birchler 2005). The activation of RISC ensures specific annealing to the complementary target RNA and the Argonaute, a catalytic component of the RISC, thereby causing the degrading of the target mRNA.

The approach encompasses using ASO to bind to and inhibit follows these assumptions: Blocking RNA splicing, hastening degradation of the RNA molecules, preventing introns from being spliced out of the mRNA, impeding the exportation of mRNA into the cytoplasm, hindering translation, the duplex formation in RNA (see Fig. 4); inhibition of 5' cap formation, alteration of the splicing process (splice-switching), and steric hindrance of ribosomal activity. The ASO can be directed towards either the initiation codon of methionine (AUG) to hinder translation or splice sites to obstruct splicing (Gupta et al. 2011; Di Fusco et al. 2019). The way ASO works seems to depend on two factors: its sequence and chemistry. The sequence determines the RNA section where the ASO will attach itself, while the chemistry controls whether it will cause RNase H cleavage or steric blockage (Eric and Cambridge 2018). Post-transcriptional processing of RNAs (pre-mRNA) is nuclear localized whereby the RNA undergoes various modifications, such as the addition of a 5' cap, splicing, and polyadenylation at the 3' regions. RNA processing regulation is an effective mechanism for controlling gene expression, and oligonucleotides can be used to achieve this (Dean and Bennett 2003; Dunoyer et al. 2006).

Methods of delivery of the asRNA into the cell

Antisense oligonucleotide (ASO) may directly or indirectly be delivered into the cell. Direct insertion involves the use of a gene gun while the indirect method may be through *Agrobacterium tumefaciens*, viral-mediated asRNA transfer or by infiltration of ASO into a cell. The indirect method through *Agrobacterium* is effective in downregulating the quantity of a specific protein (Gelvin 2003; Aregawi et al. 2022). Among the earliest studies involving the indirect approach in plants aimed to compare the effectiveness of sense, antisense, or dsRNA in generating RNA-mediated virus resistance through post-transcriptional gene silencing (PTGS) in *Nicotiana tabacum* (tobacco) (Waterhouse et al. 1998). Particle bombardment through the gene gun is an effective method of genetically transforming crops such as cereals (Hamada et al. 2018). It is particularly useful for introducing multiple genes and for transforming

antisense RNA segment of the *TOMSSSF* gene under the regulation of the cauliflower mosaic virus 35S promoter. This resulted in decreased activity of sucrose synthase (*SuSy*) in the fruits of the transgenic tomatoes (Saurabh et al. 2014; Rajput et al. 2021) and caused a delay in fruit ripening and this approach may be extended to other economical fruits like peas, mangos, banana, apples, and grapes where uncontrolled ripening may cause deterioration and high economic loss.

Virus resistance

Plant viruses are a major factor in reducing crop productivity and yield, as they can affect the quality of crops (Luckanagul et al. 2014). Antisense RNA is effective in creating resistance to plant viruses, as the antisense sequence impedes RNA biosynthesis. Day et al. (1991) reported that antisense RNA can prevent DNA viruses, the antisense DNA of the *AL 7*-coding gene from the geminivirus tomato golden mosaic virus (TGMV) was effective in suppressing TGMV replication. Pathogen-derived resistance (PDR) is a powerful approach to combat virus infections in plants and has been successfully used to engineer virus-resistant plants (Simón-Mateo and García, 2011). Inverted repeats of antisense oligonucleotide of the coat protein (CP) gene of the Tobacco Streak Virus (TSV) have been introduced as an effective and reliable strategy for developing economically important crops that are resistant to TSV (Pradeep et al. 2012). Tomato varieties that are resistant to Potato Spindle Tuber Viroid (PSTVd) were produced through antisense RNA technology (Schwind et al. 2009). Furthermore, in 1989, Powell et al. reported the development of a tobacco variety that is resistant to tobacco mosaic virus (TMV) by using the asRNA technology to modify the *CP* gene which conferred resistance to TMV.

Developing bacterial-resistant crops

Bacterial diseases pose a significant challenge to field crops, such as tomatoes, soybeans, bananas, etc. due to their fast spread and difficult control. Therefore, it is essential to develop bacterial-resistant varieties of food crops to minimize infections, reduce yield loss, and lower production costs. One promising approach is the use of asRNA to enhance bacterial resistance. In a study by Escobar et al. (2001), silencing two bacterial genes (*iaaM* and *ipt*) effectively reduced the production of crown gall tumours caused by *Agrobacterium tumefaciens* in *Arabidopsis* to nearly zero. This finding suggests that it may be possible to engineer resistance to crown gall disease in trees, ornamental plants, and even food crops (Saurabh et al. 2014).

Pest resistance

Insect pests are responsible for significant losses in crop yield and the cost of insecticides, with an estimated 20–40% reduction in yield. Despite the use of insecticides, pests have developed resistance, which is exacerbated by the high amount of synthetic pesticides used annually (Ferry et al. 2006; Gordon and Waterhouse 2007; Cagliari et al. 2019). The global expenditure on pesticides is nearly \$56 billion, with almost 6 billion pounds of chemicals used in attempts to reduce pest damage (Atwood and Paisley-Jones 2017). However, recent research has shown that unmodified antisense DNA oligonucleotides derived from the RING domain of the LdMNPV IAP-3 gene can cause high mortality rates among gipsy moth larvae, both infected and uninfected with LdMNPV (Oberemok et al. 2019). These oligonucleotides,

Challenges

One of the key drawbacks of antisense RNA technology is the challenge of effective intracellular delivery of the ASO. The ASO could bind to a non-targeted mRNA, thus the site selection in the mRNA is of great concern (Gupta et al. 2011). Therefore, antisense RNA is yet to be perfected, although the utility of small RNAs as mediators in gene silencing is of immense potential, some of the issues and controversies surrounding its use still need to be addressed with a more convincing approach (Tilahun et al. 2021). There is a need for more comprehensive research to improve the technology and develop an efficient delivery system that will enable the asRNA to bind its targeted mRNA sequence and site without any escape or off-target (Singh et al. 2020). The off-target effect could lead to false experimental inferences and interpretations, and negatively impact or alter the phenotypical expressions of the modified plants which may result in undesirable pleiotropic changes in plant morphology and development. In addition, there is a concern that the asRNA-mediated gene silencing requires specific homology between the asRNA and the targeted mRNA. Thus, long antisense oligonucleotides that could specify a unique mRNA may equally direct the RNase-H, causing it to cleave a non-target mRNA at a complementarity site (Tidd 1996). This, of course, could be counterproductive and may be used as an argument against the use of technology for crop improvement. Nevertheless, to allay the fear of off-target cleavages, the specificity of antisense action for gene silencing can be improved through some analogue modification of the backbone molecules (Fisher et al. 2002).

Outlook

There are achievements and progress recorded with the use of antisense RNA technology in crop improvement. Although, the implicit understanding of the mechanisms of antisense RNA technology is still in progress. Therefore, more antisense molecules would be subjected to scientific investigations in the future to discover the effective intercellular delivery method (s) of the ASO into the cell for the specificity of hybridization with the target mRNA. A clearer understanding of the fate of dsRNA molecules within the cytosol will enhance the utility of small RNA molecules in gene silencing. This will include a more vivid understanding of the mechanism guiding the endonucleolytic cleavage of hairpin and the precursor transcripts by RNase III-like Dicer ribonuclease and direct the cleavage of target transcripts by Argonaute RNase H-like proteins and RISC in a sequence-specific manner. In this case, more work and intensive studies are still required if the potential of the technology should be fully utilized in crop improvement to generate crop cultivars with improved agronomic traits leading to increasing yield and nutritional value towards sustainable agriculture and global food security.

Conclusion

Antisense RNA technology offers a promising way to decrease the expression of a particular gene, resulting in plants with modified traits. This technology is applicable in crop improvement in the areas of fruit ripening, enhanced shelf life, bacterial resistance, reduction of lignin content, etc. for man's benefit. However, to fully utilize the technology safely, transgenic strategies using asRNA or other small RNA

molecules should be designed only after a comprehensive understanding of the mechanism of regulation. This will minimize undesirable trade-offs in transgenic and reduce the controversies and ethical issues associated with the technology.

Declarations

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Declaration of Potential Conflicts of interest: The authors have no conflict of interest to declare whatsoever.

Data Availability Statement: No Data associated in the manuscript.

Ethical approval: This article does not contain any studies with animals performed by any of the authors.

Author Contribution

DAA and JAL developed the manuscript.

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Academic session 2023-24

Class B.Sc.- III (Sem-IV)

Project name :- DNA Fingerprinting

Submitted by –

Swaraj R. Wankhade

Guided by –

Shri. K. U. Makode

DISCOVERY OF THE DNA FINGERPRINT

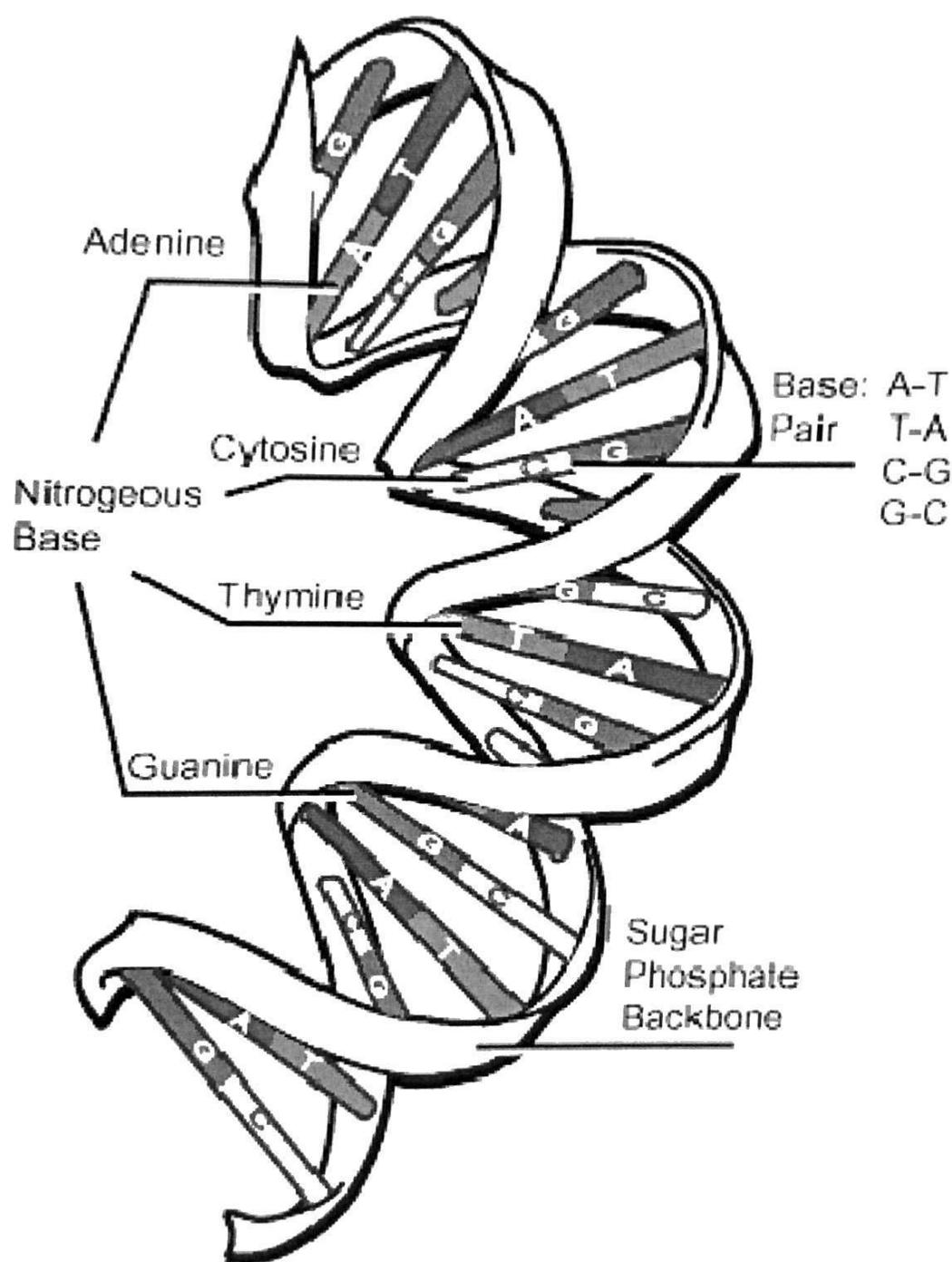
Historically, identity testing in the forensic field started with the analysis of the ABO blood group system. Later, new markers for identity and paternity identification were based on variations of serum proteins and red blood cell enzymes; eventually the human leukocyte antigen system was used. It was not until 20 years ago that Sir Alec Jeffreys, professor and geneticist at the University of Leicester in the United Kingdom (UK), pioneered DNA-based identity testing.

Professor Jeffreys was interested in studying the genetic variation between individuals and had done some of the early work to detect genetic differences in humans. However, the answer did not come to him on the initial project he was interested in but rather on an unrelated project: analysis of the myoglobin gene in seal meat at the headquarters of the British Antarctic Survey. When he and his colleagues compared the myoglobin gene in seals with the human counterpart, they found that some short repeating sequences were homologous between seals and humans. When they compared those sequences with the published sequences of tandem repeats called minisatellites, they found that they were the same. Professor Jeffreys recognized that the repeating sequences “could be highly variable, informative genetic markers”. His group developed a radioactive probe, made up of short sequences, that could latch onto those repeating sequences and ultimately reveal patterns that were unique to each individual: a DNA “fingerprint”.

The steps involved in DNA fingerprinting are as follows. First, the DNA is extracted from the specimen (i.e., blood, semen, skin, hair). After DNA extraction, restriction enzymes are added, which work like scissors to cut the DNA into the smaller segments that are different between individuals. The DNA segments are sorted by agarose gel electrophoresis and visualized by staining with ethidium bromide. A Southern blot is performed to transfer the DNA onto a membrane. A radioactive probe is applied to the membrane, and the pattern of DNA is detected by exposing the membrane to x-ray film. The result is a pattern of DNA bands that looks like a supermarket bar code. Each individual has a signature fingerprint.

Professor Jeffreys looked at a DNA fingerprint of a human family; he also looked at the fingerprint of a cow, a baboon, a mouse, and a tobacco plant. The pattern of DNA segments, composed of perhaps 15 to 20 bands, was different for each one. However, closer inspection of the patterns of the human family revealed that the mother and the father each had their own pattern and that the child had a composite of both, having inherited an allele from the father and the mother.

In the spring of 1985, Professor Jeffreys and his colleagues published their first article on DNA fingerprinting and saw the utility of it in the forensic sciences and in paternity identification. Newspapers publicized the findings, and shortly thereafter a lawyer became interested in the test and saw its applicability in one of the cases she was representing.



DNA AS A PROBLEM-SOLVING TOOL

A family from Ghana immigrated to the UK and became citizens. However, one of the sons went back to Ghana and was stopped from returning to the UK because he had a forged passport. The family's lawyer contacted Professor Jeffreys and asked whether he could confirm that the boy was in fact the mother's son and not her nephew (she had several sisters in Ghana). The situation was complicated by the absence of the father.

Samples of DNA were taken from the mother, from the son whose identity was disputed, and from the mother's three undisputed children. The patterns confirmed the relationship between the mother and the son in question. Moreover, the testing confirmed that all four children had the same father (7). This immigration case opened the door for using DNA fingerprinting in forensic cases and for identity determination.

Development of a DNA database

In the USA, the DNA Identification Act of 1994 authorized the Federal Bureau of Investigation to expand a pilot project into a national DNA database, the Combined DNA Index System (CODIS), as a tool for solving violent crimes. CODIS combines DNA analysis with computer technology to enable crime laboratories at the local, state, and national levels to exchange and compare DNA profiles electronically. The database comprises two indexes: the Forensic Index, which contains DNA profiles from crime scene evidence, and the Offender Index, which contains profiles from those convicted of felony sex offenses and other violent crimes. The system is based on the amplification of 13 core STR loci, as well as the amelogenin gene, which is located on the X and Y chromosomes and is useful in determining the sex of an individual .

Today, DNA identity testing is widely used in the field of forensics and paternity identification. Other clinical applications are based upon the methods developed for forensic testing. They include assessment of donor hematopoietic engraftment after bone marrow transplantation and chimerism analysis after solid

organ transplantation. Other uses include confirming a diagnosis of hydatidiform mole and resolving issues of specimen identity in cases of specimen mislabeling or misidentification. Finally, DNA identity testing can be used to evaluate tumor transmission after transplantation and thus determine whether a malignancy is of donor or recipient origin.

VNTR and STR are useful for identity testing because they are polymorphic and are inherited in a mendelian fashion. Each individual inherits one paternal and one maternal allele of a specific STR locus, leading to further diversity in STR pattern.

CONCLUSION

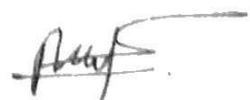
Twenty years after the development of DNA fingerprinting, DNA analysis remains the key to linking suspects to biological evidence and to identifying individuals in crimes and disasters. Another important use is the establishment of paternity in custody and child support litigation. DNA profiling is used to diagnose inherited disorders and human diseases.

The list of additional uses for DNA fingerprinting continues to grow. For example, DNA markers have proven to be powerful in the study of population genetics. Molecular markers are used to detect sudden changes in populations, effects of population fragmentation, and interaction of different populations.

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DEPARTMENT OF SCIENCE

SUBJECT :- BOTANY

Class :- BS.c III (Final Year) Sem VIth

TOPIC NAME :- Centrifuge



Centrifuges

Catalog

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Introduction

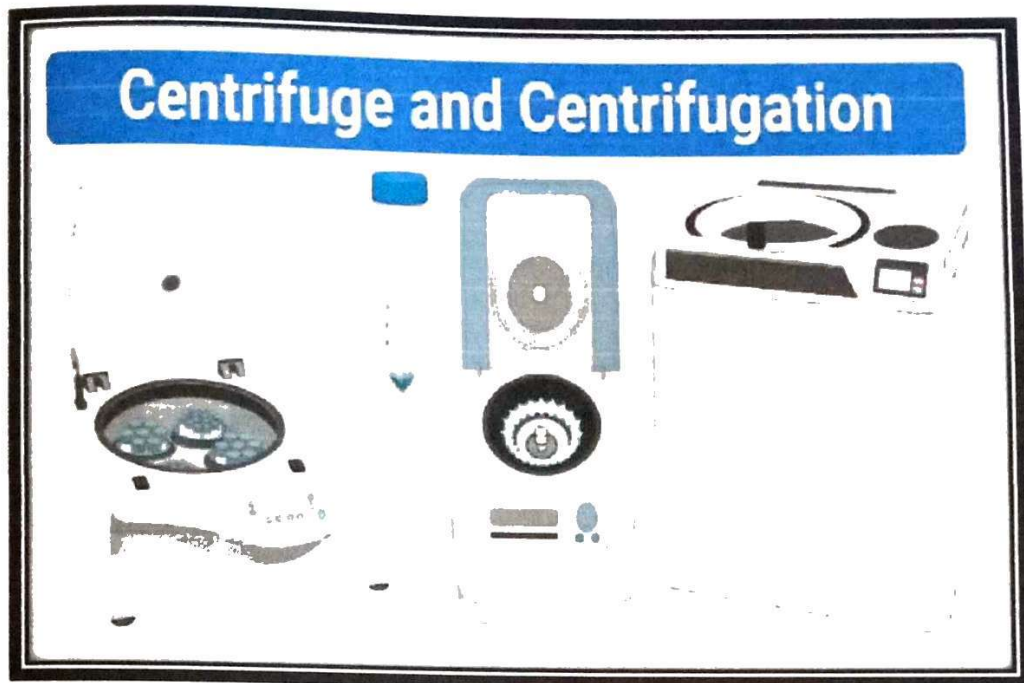
Centrifugation is a mechanical process that utilizes a spinning medium to separate one or more components of a sample according to density or size. While gaseous or immiscible liquids can be separated by centrifugation, the majority of applications involve sedimentation of solid particles in a liquid medium. Centrifugal separations may be classified as either analytical or preparative. In 'analytical centrifugation', the objective is to monitor particle sedimentation behavior in order to characterize particle properties, e.g., molecular weight, shape, and association. In 'preparative centrifugation', the objective is to separate and recover one or more components from a sample mix. Preparative centrifugation encompasses the vast majority of centrifugal applications.

Centrifuge definition

A centrifuge is a device used to separate components of a mixture on the basis of their size, density, the viscosity of the medium, and the rotor speed.

- The centrifuge is commonly used in laboratories for the separation of biological molecules from a crude extract.
- In a centrifuge, the sample is kept in a rotor that is rotated about a fixed point (axis), resulting in strong force perpendicular to the axis.

- There are different types of centrifuge used for the separation of different molecules, but they all work on the principle of sedimentation.



Relative Centrifugal Force (RCF)

- Relative centrifugal force is the measure of the strength of rotors of different types and sizes.
- This is the force exerted on the contents of the rotor as a result of the rotation.
- RCF is the perpendicular force acting on the sample that is always relative to the gravity of the earth.
- The RCF of the different centrifuge can be used for the comparison of rotors, allowing the selection of the best centrifuge for a particular function.

The formula to calculate the relative centrifugal force (RCF) can be written as:

$$\text{RCF (g Force)} = 1.118 \times 10^{-5} \times r \times (\text{RPM})^2$$

where **r** is the radius of the rotor (in centimeters), and **RPM** is the speed of the rotor in rotation per minute.

Types of centrifuges

- 1. Benchtop centrifuge***
- 2. Continuous flow centrifuge***
- 3. Gas centrifuge***
- 4. Hematocrit centrifuge***
- 5. High-speed centrifuge***
- 6. Low-speed centrifuge***
- 7. Microcentrifuge***
- 8. Refrigerated centrifuges***
- 9. Ultracentrifuges***

Types of centrifugation

- 1. Analytical Centrifugation**

Principle of Analytical Centrifugation

- Analytical centrifugation is based on the principle that particles that are denser than others settle down faster. Similarly, the larger

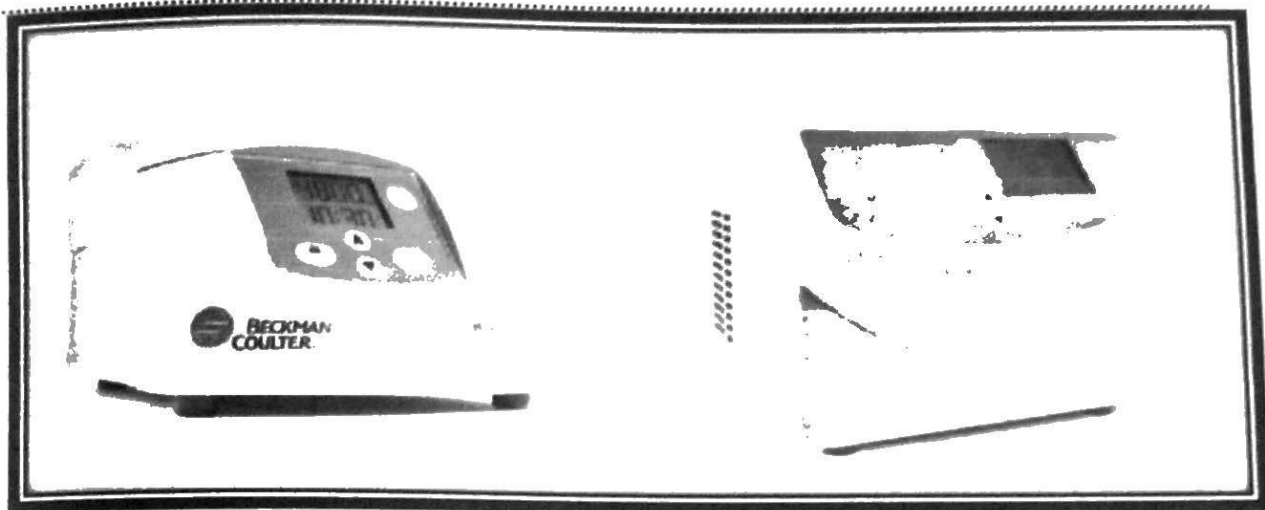
molecules move more quickly in the centrifugal force than the smaller ones.

- Analytical ultracentrifugation for the determination of the relative molecular mass of a macromolecule can be performed by a sedimentation velocity approach or sedimentation equilibrium methodology.
- The hydrodynamic properties of macromolecules are described by their sedimentation coefficients. They can be determined from the rate that a concentration boundary of the particular biomolecules moves in the gravitational field.
- The sedimentation coefficient can be used to characterize changes in the size and shape of macromolecules with changing experimental conditions.
- Three optical systems are available for the analytical ultracentrifuge (absorbance, interference, and fluorescence) that permit precise and selective observation of sedimentation in real-time.

Uses of Analytical Centrifugation

- Analytical centrifugation can be used for the determination of the purity of macromolecules.

- It can also be used for the examination of changes in the molecular mass of supramolecular complexes.
- Besides, it allows the determination of the relative molecular mass of solutes in their native state.



2. Density gradient centrifugation

Density gradient centrifugation is the separation of molecules where the separation is based on the density of the molecules as they pass through a density gradient under a centrifugal force.

Principle of Density gradient centrifugation

- Density gradient centrifugation is based on the principle that molecules settle down under a centrifugal force until they reach a medium with the density the same as theirs.
- In this case, a medium with a density gradient is employed, which either has to decrease density or increasing density.
- Molecules in a sample move through the medium as the sample is rotated creating a centrifugal force.

- Further particles settle down depending upon the speed and time of individual centrifugation steps and the density and relative size of the particles.
- The largest class of particles forms a pellet on the bottom of the centrifuge tube, leaving smaller-sized structures within the supernatant.
- Thus, larger molecules sediment quickly and at lower centrifugal forces whereas the smaller molecules take longer time and higher forces.
- In the case of particles that are less dense than the medium, the particles will float instead of settling.

Uses of Differential centrifugation

- Differential centrifugation is commonly used for the separation of cell organelles and membranes found in the cell.
- It can also be used for low-resolution separation of the nucleus.
- As this technique separates particles based on their sizes, this can be used for the purification of extracts containing larger-sized impurities.

4. Isopycnic centrifugation

Isopycnic centrifugation is a type of centrifugation where the particles in a sample are separated on the basis of their densities as centrifugal force is applied to the sample.

Principle of Isopycnic centrifugation

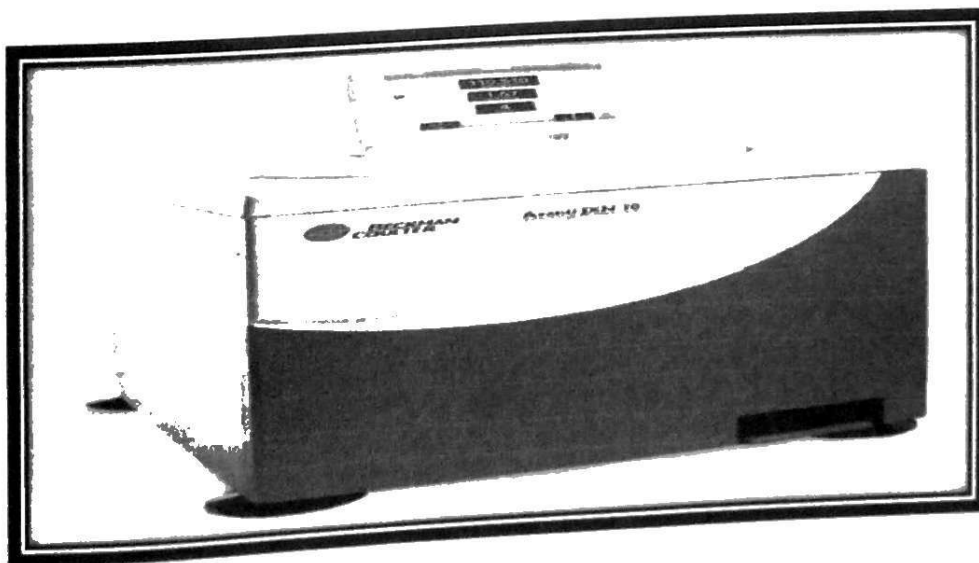
- Isopycnic centrifugation is also termed the equilibrium centrifugation as the separation of particles takes place solely on the basis of their densities and not on their sizes.
- The particles move towards the bottom, and the movement is based on the size of the particles. And, the flow ceases once the density of the particle becomes equal to the density of the surrounding medium.
- The density in the gradient increases as we move down the tube towards the bottom. As a result, the particles with higher densities settle down at the bottom, followed by less dense particles that form bands above the denser particles.
- It is considered as a true equilibrium as this depends directly on the buoyant densities and not the sizes of the particles.

5. Rate-zonal density gradient centrifugation/ Moving Zone Centrifugation

Rate-zonal density gradient centrifugation is a type of centrifugation that separates particles on the basis of their shape as size and works on the same principle of density gradient centrifugation but works in a different way. It is also called the moving zone centrifugation.

Principle of Rate-zonal density gradient centrifugation

- Rate zonal centrifugation fractionates particles by both size and shape.
- The procedure is to layer a sample in a restricted zone on top of a pre-poured density gradient. The density gradient is then centrifuged.
- All particles migrate into the density gradient because the density gradient has only densities much lower than the densities of the particles being centrifuged.
- The particles are fractionated primarily by size and shape. The larger a particle is, the more rapidly it sediments.
- The more spherically symmetrical a particle is, the more rapidly it sediments.
- The particles sediment through the gradient at a rate that is a function of their sedimentation coefficient.
- Unlike differential centrifugation where the sample is distributed throughout the medium, in rate-zonal centrifugation, the sample is initially present only on top of the gradient as a narrow band.



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DEPARTMENT OF BOTANY

SESSION : 2023-2024

B.SC-III (SEM-VI)

SUMMER – 2024

A PROJECT REPORT ON

TRANSGENIC TOMATO

Submitted By

Mr. Shaikh Awais Shaikh Azeez

Guided By

Dr. Keshav Makode

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DEPARTMENT OF BOTANY

SESSION : 2023-2024

CERTIFICATE

**This is certify that Mr. Shaikh Awais
Shaikh Azeez. Student of B.Sc 3rd Year
(Semester 6th) has successfully
completed the research on the project
TRANSGENIC TOMATO. Under the
guidance of Dr. Keshav Makode
(Department Of Botany) during the year
of session 2023 – 2024.**

Teacher's Sign

Signature Of HOD

INTRODUCTION:

A person living in a Westernized culture often takes for granted the hard work, resource usage, and waste that occur to bring food to him. Tomatoes, for example, currently follow a long and difficult route to the supermarket. To begin with, field workers must pick the tomatoes by hand while they are still green. The unripe tomatoes are then trucked to facilities where they are gassed with ethylene to artificially induce ripening. Normal tomatoes grown commercially cannot be allowed to ripen on the vine because they soften during the ripening process. Picking them while they are still hard allows them to be shipped, but it also prevents the development of natural flavors. Therefore, supermarket tomatoes generally have little flavor. Tomato, potato, squash and papaya are among a variety of crops that have been modified to resist infection by viruses or insect pests. Plant productivity is influenced by abiotic factors such as herbicides, soil composition, water supply, and temperature. Therefore, conferring plants with genes that will help them withstand a wider range of environmental conditions could increase productivity. Plants are also being genetically modified to withstand drought, heat, cold temperatures and poor soil conditions such as salinity and aluminum contamination. Increased productivity also can be achieved by enhancing efficiencies in the metabolic and photosynthetic pathways. Some pathways that could be improved to increase crop yield include nitrogen assimilation, starch biosynthesis, and modification of photosynthesis. Traditionally to prevent delivering spoiled fruit, mature tomatoes are harvested while still green and ripened during delivery by exposure to ethylene, a ripening hormone in tomatoes. In 1994 the Food and Drug Administration approved a brand of tomato that had a genetic solution to this processing problem. To utilize potentially fertile land, scientists have been trying to use new tools provided by molecular biology to see if plants could be genetically engineered to grow well in saline soils.

METHODS OF TRANSFORMATION:

There are various methods of transformation available which are followed by different laboratories. To create the transgenic tomato, a gene from *E. coli* (a bacterium which occurs naturally in the mammalian gut) called *kan(r)* and the FLAVR SAVR gene (from a tomato) were inserted into a plasmid (a circular ring of DNA) and plasmids like these were inserted into a group of tomato cells in a growth medium containing an antibiotic. The *Kan (r)* gene, when established in the cell, produced a substance called APH (3') II that gave the cell resistance to the antibiotic. The antibiotic killed cells that did not receive the plasmid. The purpose of the bacterial gene was, therefore, to identify the cells that were genetically transformed. The FLAVR SAVR gene coded for a strand of RNA that was the reverse of a strand of RNA that naturally occurs in the plant. The original RNA strand in the plant is responsible for the production of the enzyme polygalacturonase. Polygalacturonase breaks down pectin in the cell walls of the tomato during the ripening process and causes the entire tomato to become soft. The complementary strand of RNA from the FLAVR SAVR gene binds to the polygalacturonase RNA and the two strands "cancel each other out," preventing the production of polygalacturonase and the softening of the tomato.

Tomato transformation and regeneration were analyzed and optimized by Carolina Cortina and coworkers. They infected Cotyledon explants from *Lycopersicon esculentum* cv. UC82B with *Agrobacterium tumefaciens* strain LBA4404 harbouring the neomycin phosphotransferase (NPTII) reporter gene. They found that on increasing concentration of thiamine(vitamin) from 0.1 mg l⁻¹ in standard medium to 0.4 mg l⁻¹ decreased the chlorophyll lost that accompanied the expansion of necrotic areas in cotyledon explants.

They observed Optimal shoot regeneration rate with a balanced concentration of 0.5 mg l⁻¹ auxin indolelactic acid (IAA) and 0.5 mg l⁻¹ cytokinin zeatin riboside. Finally, when the phenolic acetosyringone was present in the co-culture medium at 200 µM, they confirmed transgenic lines reached 50% of antibiotic resistant shoots. The efficiency of transformation reached 12.5% with this protocol. Chyi Y.S. and coworkers investigated the genetic behavior of DNA sequences in the backcross progeny of 10 transformed *Lycopersicon esculentum* x *L. pennellii* hybrids. They used Isozyme and restriction fragment length polymorphism (RFLP) markers to test linkage relationships of the insertion in each backcross family. The TDNA inserts in 9 of the 10 transformants were mapped in relation to one or more of these markers, and each mapped to a different chromosomal location. Because only one insertion did not show linkage with the markers employed, it must be located somewhere other than the genomic regions covered by the markers assayed. They concluded that *Agrobacterium*-mediated insertion in the *Lycopersicon* genome appears to be random at the chromosomal level. Backcross progeny of two nopaline negative transformants showed incomplete correspondence between the T-DNA genotype and the kanamycin resistance phenotype. Two kanamycin resistant progeny plants of one of these two transformants possessed altered T-DNA restriction patterns, indicated genetic instability of the T-DNA in this transformant McCormick S. modified leaf disc transformation system in tomato. They used leaf explants and hypocotyls sections can be used to regenerate transformed plants. They found evidences for both single and multicopy insertions of the T-DNA, and have demonstrated inheritance of the T-DNA insert in the expected Mendelian ratios. A reduced efficiency of transformation was observed with binary T-DNA vectors as compared to co-integrate T-DNA vectors.

Table 1:

Comparison of Ranges of Nutrients between Transgenic and Normal Tomatoes (per 100 g Fruit).

Nutrient	Normal Range	Transgenics	Controls
Protein	0.85 g	0.75 - 1.14 g	0.53 - 1.05 g
Vitamin A	192 - 1667 IU	330 - 1660 IU	420 - 2200 IU
Thiamin	16 – 80 µg	38 – 72 µg	39 – 64 µg
Riboflavin	20 – 78 µg	24 – 36 µg	24 – 36 µg
Vitamin B6	50 – 150 µg	86 – 150 µg	10 – 140 µg
Vitamin C	8.4 – 59 mg	15.3 – 29.2 mg	12.3 – 29.2 mg
Niacin	0.3 – 0.85 mg	0.43 – 0.76 mg	0.43 – 0.76 mg
Calcium	4.0 – 21 mg	9 – 13 mg	10 – 12 mg
Phosphorus	7.7 – 53 mg	25 – 37 mg	29 – 38 mg
Sodium	1.2 – 32.7 mg	2 – 5 mg	2 – 3 mg

Note:

For Table 1, the "Normal Range" represents values that the researchers looked up in standard references. The "Controls" column represents actual amounts of nutrients found in nontransgenic (traditional) varieties grown by the researchers alongside the transgenic varieties.

tumefaciens through triparental mating. Cotyledon leaf explants of Pusa Ruby tomato were transformed by co-cultivation with Agrobacterium containing TLCV–CP constructs. Kanamycin-resistant transformants were regenerated and established in glasshouse. They observed that in T1 generation transformed plants showed disease tolerance when compared to non transformed ones.

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**Department of Botany
Session 2023-24
Class- B. Sc III Semester VI (CBZ)**

**A project report
On**

Gene Gun

Submitted by

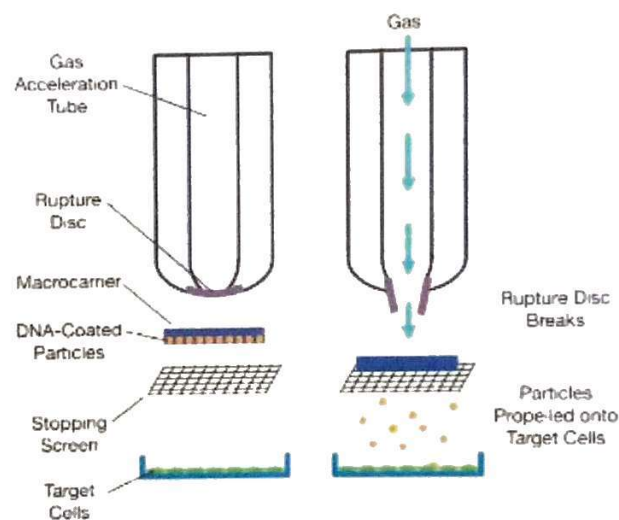
Miss: Jaweriya Firdous Mohammad Ismail

Guided by

Prof: Shri Keshav Makode

Introduction:

The particle bombardment, also known as the gene gun, is a direct physical method of gene delivery (introducing nucleic acids into cells). It is a device used to transfect cells with foreign DNA by bombarding the target cells with DNA-coated microparticles. In this method, nucleic acids or other biological molecules are coated onto high-density gold or tungsten microparticles (microcarriers), then they are accelerated to high velocity by a helium pulse and driven through cell walls and membranes into the target (Fig.1). The gene gun was originally developed to deliver genetic materials into plant cells. Today, this technique has been used to transfer DNA coated micro-particles to achieve gene transfection into many types of cells and organs, including cell cultures, tissues, organs, plants, animals, fungi and bacteria, as well as organelles.



Definition:

A method of transferring large molecules such as DNA into the cell by coupling the DNA to a nanoparticle of an inert solid (typically gold) to be injected into the nucleus of the target cell. Supplement Molecules such as DNA, RNA, proteins, and others can be moved into the target cell. The process is called transfection. It is carried out in order for those molecules to be delivered into the semi impermeable plasma membrane of the cell. The possible applications of transfection are for therapeutic purposes (i.e. gene therapy) or for research. Several methods are used nowadays for transfection. One of them is the gene gun. It is regarded as a biolistic particle delivery system because the nanoparticle of an inert solid (e.g. gold) carrying the target gene on its surface is fired into the target cell at very high speeds. Gene guns are originally used for transfecting plant cells. However, Gene guns can also be used in human and animal cells. For instance, gene gun can be used to deliver DNA vaccine. It is also used in labeling subsets of cells in cultured tissue.

Gene gun:

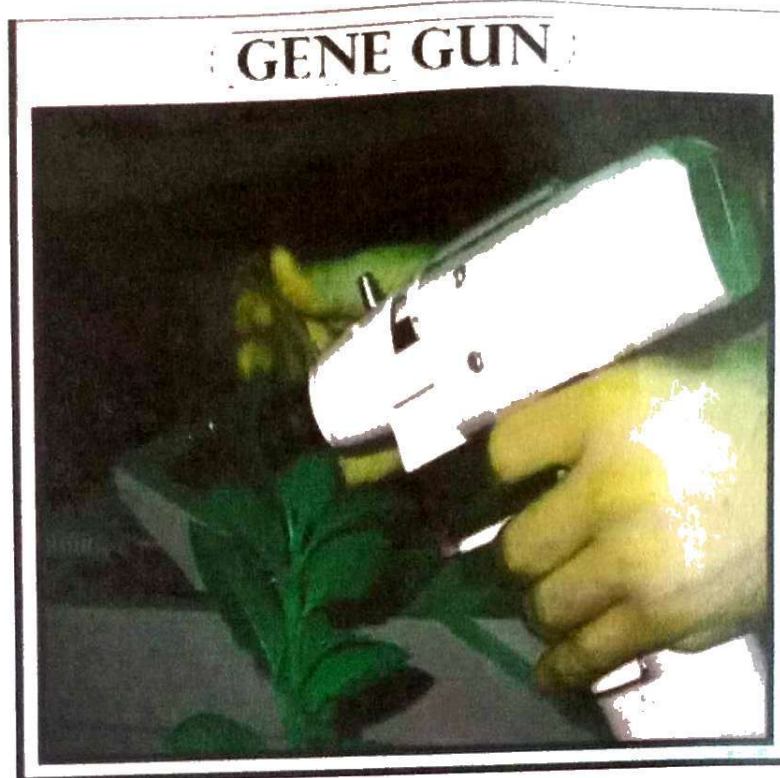
In genetic engineering, a gene gun or biolistic particle delivery system is a device used to deliver exogenous DNA (transgenes), RNA, or protein to cells. By coating particles of a heavy metal with a gene of interest and firing these micro-projectiles into cells using mechanical force, an integration of desired genetic information can be introduced into desired cells. The technique involved with such micro-projectile delivery of DNA is often referred to as biolistics, short for "biological ballistics".



Gene Gun Method:

Gene gun method is a method of transfecting cells by bombarding them with microprojectiles coated with DNA. It is a kind of biolistic (bioballistic). Under certain conditions, DNA (or RNA) becomes "sticky," adhering to biologically inert particles such as metal atoms (usually tungsten or gold). By accelerating this DNA-particle complex in a partial vacuum and target tissue within the acceleration path, DNA is effectively introduced (Gan, 1989). Uncoated metal particles could also be shot through a solution containing DNA surrounding the cell thus picking up the genetic material and proceeding into the living cell. A perforated plate stops the shell cartridge but allows the slivers of metal to pass through and into the living cells on the other side. The cells that take up the desired DNA, identified through the use of a marker gene (in plants the use of GUS is most common), are then cultured to replicate the gene and possibly cloned. The biolistic method is most useful for inserting genes into plant cells such as pesticide or herbicide resistance. Different methods have been used to accelerate the particles: these include pneumatic devices; instruments utilizing a mechanical impulse or macroprojectile; centripetal, magnetic or electrostatic forces; spray or vaccination guns; and apparatus based on acceleration by shock wave, such as electric discharge. This technique has been used successfully to transform soybean, cotton, spruce, sugarcane, papaya, corn, sunflower, rice, maize, wheat, tobacco etc. Genomes of subcellular organelles have been accessible to genetic manipulation by biolistic method. Mitochondria of plants and chloroplast of *Chlamydomonas* have been transformed. Method can be applied to filamentous fungi and yeast (mitochondria). The particle gun has also been used with pollen, early stage embryoids, meristems and somatic embryos.

The use of the gene gun may be contrasted with the use of *Agrobacterium tumefaciens* and its Ti plasmid to insert DNA into plant cells. See transformation for different methods of transformation in different species.



3. Other Biological Systems:

Biolistic technology has been applied to various targets, such as chloroplasts, mitochondria, bacteria, fungi, algae, and pollen. Today, the gene gun is used for genetic transformation of many organisms to introduce a diverse range of desirable traits. For example, particle bombardment plays an important role in the transformation of organelles such as chloroplasts, which enables engineering of organelle-encoded herbicide or pesticide resistance in crop plants and to study photosynthetic processes. In addition, gene guns have been further used to study cancer, infectious diseases and wound healing, to generate immune responses in animals and measure gene expression and regulation in vivo and in vitro in recent years.

Advantages Of gene gun:

Biolistics has proven to be a versatile method of genetic modification and it is generally preferred to engineer transformation-resistant crops, such as cereals. Notably, Bt maize is a product of biolistics.[9][page needed] Plastid transformation has also seen great success with particle bombardment when compared to other current techniques, such as *Agrobacterium* mediated transformation, which have difficulty targeting the vector to and stably expressing in the chloroplast.[9][page needed][18] In addition, there are no reports of a chloroplast silencing a transgene inserted with a gene gun.[19] Additionally, with only one firing of a gene gun, a skilled technician can generate two transformed organisms in certain species.[18] This technology has even allowed for modification of specific tissues in situ, although this is likely to damage large numbers of cells and transform only some, rather than all, cells of the tissue.

- This method is easy to use, fast and versatile.
- Effective transformation requires only a small amount of nucleic acid and few cells
- High levels of co-transformation and codelivery of multiple plasmids are possible.
- Can transfer large DNA fragments as well as small interfering RNA (siRNA) for gene silencing.
- Can transfect many cell types, including non-dividing cells and plant cells.
- Avoid potentially toxic treatments (such as from viruses or chemical and lipid-based systems).

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