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Advances in Plant Science

Volume I

Editors

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PREFACE

We are delighted to publish our book entitled ***"Advances in Plant Science Volume I".*** *This book is the compilation of esteemed articles of acknowledged experts in the fields of plant science providing a sufficient depth of the subject to satisfy the need of a level which will be comprehensive and interesting. lt is an assemblage of variety of information about advances and developments in plant science. With its application oriented and interdisciplinary approach, we hope that the students, teachers, researchers, scientists and policy makers will find this book much more useful.*

The articles in the book have been contributed by eminent scientists, academicians. Our special thanks and appreciation goes to experts and research workers whose contributions have enriched this book. We thank our publisher Bhumi Publishing, lndia for compilation of such nice data in the form of this book.

Finally, we will always remain a debtor to all our well-wishers for their blessings, without which this book would not have come into existence.

- ***Editors***
- ***Advances in Plant Science Volume I***

CONTENTS

A STUDY ON EFFICACY OF PGPR ISOLATED FROM ROOT NODULES AND RHIZOSPHERE SOIL

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Abstract:

The bacterial strains were isolated from root nodules of Cowpea (*Vigna ungiculata)* and its rhizhosphere soil. The bacterial strains such as *Rhizobium* sp., *Azotobacter* sp. and *Derxia* sp. were identified using morphological and biochemical charecteristics. The identified bacterial strains such as *Rhizobium* sp., *Azotobacter* sp. and *Derxia* sp. were screened for IAA production. Then the plant growth promoting activity of three isolated bacterial strains such as *Rhizobium* sp., *Azotobacter* sp., *Derxia* sp. and mixed culture (*Rhizobium* sp, *Azotobacter* sp. and *Derxia* sp.) culture broth was tested at different concentrations (1,3 and 5ml) and at different days (5^{th} 10^{th} and 15^{th}) intervals. The growth parameters such as root length (10.66±0.08) shoot length (21.10±0.11), fresh weight (9.60±0.15) and dry weight (0.96±0.02) have been enhanced by mixed culture than seeds treated with single cultures.

Keywords: Root nodules, rhizosphere soil, PGPR

Introduction:

Microorganisms in the soil are essential for the maintenance and sustainability of plant and animal communities by enabling the transformation of nutrients and by performing many key biological processes. The organic matter released by plant roots increases the microbial activity around the roots, where a large number of microscopically organisms, such as bacteria, fungi, protozoa and algae coexist. The ‘rhizosphere’ is a term first used to describe this locality as ‘the compartment influenced by the roots’ in which intense interactions between beneficial and pathogenic microorganisms occur at all times. While the bacteria utilize the nutrients that are released from the host for their growth, they also secrete a wide variety of metabolites into the

rhizosphere. Root colonizing plant-beneficial bacteria is called plant. Climate condition and modern agriculture are severely modifying and polluting the natural environment. Rhizo bacteria that exert beneficial effects on plants called "plant growth – promoting rhizo bacteria" (PGPR) are alternative to the use of chemicals. PGPR may benefit the host by causing plant growth promotion or biological disease control. PGPR activity has been reported in strains belonging to several genera, such as *Azotobacter* sp, *Azospirillum* sp, *Pseudomonas* sp, *Acetobacter* sp, *Burkholderia* sp. and *Bacillus* sp. by Kloepper (2004), Kloepper and Schroth (1989) and Glick and Bashan (1997).

Methodology:

Collection of soil sample:

Soil sample from the rhizosphere of cowpea (*Vigna ungicualta*) plants were collected from Rajapalayam, Virudhunagar District, Tamil Nadu, India during December month.

Identification of bacterial isolates:

Gram staining, motility test, catalase test, indole production test, methyl red test Voges Prousker test, citrate utilization test, nitrate reductase test, urease test, starch hydrolysis, glucose utilization test and hydrogen sulphide production test were done using the standard methods.

Screening of Bacterial Isolates for Indole-3-Aetic Acid (IAA) production:

The organisms isolated form rhizosphere region was identified and they were screened for their ability to produce IAA. Test bacterial culture was inoculated in the respective medium with tryptophan and incubated at 37^0C for 2-3 days. After incubation period cultures were centrifuged at 8000rpm at 30 minutes. The supernatant was collected and mixed with 2 drops of orhophosphoric acid and 4ml Salkowoski reagent. (50 ml of 35% Perchloric acid, 1 ml of 0.5 gm Ferric chloride). Incubate the tubes in dark room for half an hour. The tubes showed pink color indicates positive result for IAA. Then the OD was measured at 530nm. The results were plotted in graph and they were compared with standard IAA.

Standard IAA Preparation:

0.5g of indole-3-acetic acid is mixed with 100ml of distilled water. From this standard take (0.5, 1, 2, 3, 4, 5ml) various concentration up to 10ml for each concentration. Read the values using spectrophotometer at 530 nm. Optical density was noted and plotted in the standard graph.

Mass Multiplication:

The pure culture of selected bacterial strain was grown in selective broth, *Rhizobium* sp., *Azotobacter* sp. and *Derxia* sp. nutrient broth with tryptophan. This was called starter culture.

Then they were added in appropriate broth mass multiplication. And the broth was incubated at 7 days.

Seed Germination study

Seedling bioassay was done using *Rhizobium* sp. nutrient broth (YEMA), *Azatobactor* sp. nutrient broth (Wakshman medium), *Dexria* sp. nutrient broth (*Dexria* sp.) for test organism (culture) supernatant was used for cup culture studies using cowpea (*Vigna unguiculata* L. walp) at various concentration (1ml, 3ml, 5ml) at different day interval (5 day, 10 day,15 day). Growth parameter such as root length, shoot length, leaf area index, dry weight of the whole plant, fresh weight of the whole plant.

Results and Discussion:

Isolation andidentification of bacterial strains:

The bacterial strains were isolated from the rhizosphere soil and the root nodules. Among them three predominant colonies was chosen for further studies. The bacterial strain was based on the colony morphology, gram staining and other biochemical test.

Screening of bacterial strains for IAA production:

Bacterial isolates screened for IAA production were grown in nutrient medium supplemented with L-tryptophan were centrifuged at 10,000 rpm for 15 min. 2 ml of supernatant was mixed with 1-2 drops of Orthophosphoric acid and 4ml of Salkowskireagent. The bacterial strain *(Rhizobium sp. Azotobactor sp. and Derxia*. sp.) showed pink color development.

Plant growth promoting activity of the bacterial isolates:

The cow pea seeds were surface sterilized using 5% mercuric chloride solution for 2-3 min. Germination percentage of *(Vigna unguiculata*) seeds sown in vermiculate supplemented with *Rhizobium sp., Azotobactor sp., Derxia* sp. mixed culture broth given in table 1, 2 and table 3.

The global necessity to increase agricultural production from a steadily decreasing I degrading l and resource base has placed considerable strain on the fragile agro systems. Current strategies to maintain and improve agricultural productivity via high II practices places considerable emphasis on ‘failsafe’ techniques for each component of I production sequence with little consideration to the integration of these components in a holistic, systems approach. While the use of mineral fertilizers is considered the quickest and surest way of boosting crop production, their cost and other constraints deter farmers from ping them in recommended quantities.

Table 1: Evaluation of root length, shoot length, fresh and dry weight of Cow pea grown in **vermiculite supplemented with *mixed* (*Rhizobium sp.*, *Azotobactor sp.*, *Derxia* sp. culture broth) at three different concentrations (1, 3 and 5ml) on 5 day and in the control**

Sr. No	Concentration	Plant growth parameters			
		Root length (cm)	Shoot length (cm)	Fresh weight (g)	Dry weight (g)
1	Control	3.23±0.08	4.73±0.23	2.00±0.01	0.20±0.00
2	1ml	4.90±0.57	7.26±0.03	2.30±0.01	0.23±0.04
3	3ml	5.06±0.12	7.70±0.57	2.90±0.01	0.29±0.00
4	5 ml	5.90±0.05	8.10±0.11	3.00±0.01	0.30±0.00

Table 2: Evaluation of root length, shoot length, fresh and dry weight of Cow pea grown in vermiculite supplemented with. *Mixed* (*Rhizobium sp.*, *Azotobactor sp.*, *Derxia* sp. culture broth) at three different concentration (1, 3 and 5ml) on 10 day and in the control

Sr. No	Concentration	Plant growth parameters			
		Root length (cm)	Shoot length (cm)	Fresh weight (g)	Dry weight (g)
1	Control	5.50±0.11	8.86±0.08	3.10±0.02	0.31±0.01
2	1ml	8.46±0.08	12.93±0.03	4.10±0.01	0.41±0.02
3	3ml	8.73±0.88	13.0±0.06	5.60±0.01	0.56±0.03
4	5ml	9.26±0.08	13.26±0.08	5.90±0.01	0.59±0.02

Table 3: Evaluation of root length, shoot length, fresh and dry weight of Cow pea grown in vermiculite supplemented with. *Mixed* (*Rhizobium sp.*, *Azotobactor sp.*, *Derxia* sp. culture broth) at three different concentration (1, 3 and 5ml) on 15 day and in the control

Sr. No	Concentration	Plant growth parameters			
		Root length (cm)	Shoot length (cm)	Fresh weight (g)	Dry weight (g)
1	Control	7.70±0.15	13.80±0.17	4.20±0.02	0.42±0.01
2	1ml	10.10±0.11	20.36±0.88	5.60±0.01	0.56±0.02
3	3ml	10.26±0.88	20.76±0.88	6.80±0.02	0.68±0.01
4	5 ml	10.66±0.08	21.10±0.11	9.60±0.15	0.96±0.03

In recent years, concepts of integrated plant nutrient management (IPNM) have been developed, which emphasize maintaining and increasing soil fertility by optimizing all possible sources (organic and inorganic) of plant nutrients required for crop growth and quality. This is done in an integrated manner appropriate to each popping system and farming situation. Improvement in agricultural sustainability requires optimal use and management of soil fertility and soil physical properties, both the soil biological processes and soil biodiversity. An understanding of microbial diversity respective in agricultural context is important and useful to arrive at measures that can act indicators of soil quality and plant productivity. In this context, the long-lasting challenges soil microbiology is development of effective methods to know the types of microorganisms present in soils, and to determine functions which the microbes perform. The importance of plant growth regulators in plant tissue culture is well documented. Phyto technology offers a broad range of plant growth regulators specifically tested for plant cell culture. Each product is assayed for physical and chemical characteristics then is biologically tested following the criteria established for powdered media. Each auxin is tested for enhancement of callus growth and/ or root initiation in vitro. Each cytokinin is tested for stimulation of shoot production by Arshad and Frankenberger (1998).

In 1 ml concentration of mixed culture (*Rhizobium* sp., *Azotobacter* sp., *Derxia* sp.) the vegetative growth parameters such as root length (4.90± 0.57), shoot length (7.26±0.03), fresh weight (2.30±0.01) and dry weight (0.23±0.01) were higher than the other isolated organisms. *Rhizobium* sp., *Azotobacter* sp., *Derxia* sp. respectively on 5th day such as *Rhizobium* sp, root length (4.73±0.12) shoots length (7.16±0.03), fresh weight (1.60±0.02) and dry weight (0.16±0.01). Whereas in *Azotobacter* sp. root length (4.30±0.05) shoot length (6.93±0.03), fresh weight (1.5±0.02) and dry weight (0.15±0.03) were lesser than the values promoted by *Rhizobium* sp., *Dexria* sp. root length (4.00±0.05) shoot length (6.20±0.15), fresh weight (1.20±0.15), and dry weight (0.12±0.04) whereas in 3 ml of mixed culture (*Rhizobium* sp., *Azotobacter* sp., *Derxia* sp.) and isolated organism studies also the vegetative growth was highly promoted in mixed culture, root length (5.06±0.12), shoot length (7.70±0.57) fresh weight (2.90±0.01) dry weight (0.29±0.01). *Rhizobium* sp. root length (5.00±0.05) shoots length (7.50±0.57), fresh weight (2±0.01) and dry weight (0.20±0.01). *Azotobacter* sp. vegetative growth values were root length (4.40±0.05) shoot length (7.13±0.08), fresh weight (1.80±0.01) and dry weight (0.18±0.01). *Derxia* sp. showed lesser vegetative growth promotion such as root length (4.10±0.05), shoot length (6.46±0.08), fresh weight (1.40±0.01) and dry weight (0.14±0.02). In 5 ml concentration of plant growth studies using mixed culture (*Rhizobium* sp., *Azotobacter* sp., *Derxia* sp.) and isolated organism studies also the vegetative growth was highly

promoted in mixed culture, root length (5.90±0.05), shoot length (8.10±0.11) fresh weight (3.00±0.01) dry weight (0.30±0.04). The values vegetative growth parameters promote by *Rhizobium* sp. were root length (5.56±0.08) shoot length (7.76±0.13), fresh weight (2.30±0.01) and dry weight (0.23±0.03). Similarity vegetative growth promoted by *Azotobacter* sp. was root length (4.70±0.05), shoot length (7.15±0.08), fresh weight (2.00±0.01) and dry weight (0.2±0.00). *Derxia* sp. showed lesser vegetative growth promotion such as root length (4.1±0.05), shoot length (6.466±0.08), fresh weight (1.60±0.01) and dry weight (0.16±0.03) Cassan *et al.* (2009).

In 1 ml concentration of mixed culture (*Rhizobium* sp., *Azotobacter* sp., *Derxia* sp.) the vegetative growth parameters such as root length (8.46±0.08), shoot length (12.93±0.03), fresh weight (4.10±0.01) and dry weight (0.41±0.03) were higher than the other isolated organisms. *Rhizobium* sp., *Azotobacter* sp., *Derxia* sp. respectively on 10^{th} day such as *Rhizobium* sp., root length (8.03±0.08) shoots length (12.88±0.08), fresh weight (2.40±0.02) and dry weight (0.24±0.01). Whereas in *Azotobacter* sp. root length (8.00±0.05) shoot length (12.43±0.03), fresh weight (2.8±0.01) and dry weight (0.28±0.07) were lesser than the values of *Rhizobium* sp., *Dexria* sp. root length (7.76±0.03) shoot length (11.83±0.06), fresh weight (2.40±0.01), and dry weight (0.24±0.03) whereas in 3 ml of mixed culture (*Rhizobium* sp., *Azotobacter* sp., *Derxia* sp.) and isolated organism studies also the vegetative growth was highly promoted in mixed culture, root length (8.73±0.88), shoot length (13.03±0.06) fresh weight (5.60±0.01) dry weight (0.56±0.02). *Rhizobium* sp. root length (8.26±0.06) shoots length (12.93±0.08), fresh weight (3.00±0.02) and dry weight (0.30±0.01). *Azotobacter* sp. vegetative growth values were root length (8.13±0.03) shoot length (12.50±0.05), fresh weight (3.40±0.01) and dry weight (0.34±0.02). *Derxia* sp. showed lesser vegetative growth promotion such as root length (4.10±0.05), shoot length (6.46±0.08), fresh weight (3.60±0.01) and dry weight (0.36±0.02). In 5 ml concentration of plant growth studies using mixed culture (*Rhizobium* sp., *Azotobacter* sp., *Derxia* sp.) and isolated organism studies also the vegetative growth was highly promoted in mixed culture, root length (9.26±0.08), shoot length (13.26±0.08) fresh weight (6.70±0.01) dry weight (0.67±0.02). The values vegetative growth parameters promote by *Rhizobium* sp. were root length (8.40±0.05) shoot length (13.10±0.01), fresh weight (3.50±0.05) and dry weight (0.35±0.02). Similarity vegetative growth promoted by *Azotobacter* sp. was root length (8.20±0.05), shoot length (12.66±0.08), fresh weight (4.60±0.01) and dry weight (0.46±0.04). *Derxia* sp. showed lesser vegetative growth promotion such as root length (8.03±0.08), shoot length (12.36±0.08), fresh weight (4±0.01) and dry weight (0.40±0.01) Gaskins *et al.* (1985).

In 1 ml concentration of mixed culture (*Rhizobium* sp., *Azotobacter* sp., *Derxia* sp*)* the vegetative growth parameters such as root length (10.10± 0.11), shoot length (20.36±0.88), fresh

weight (5.60±0.01) and dry weight (0.56±0.01) were higher than the other isolated organisms. *Rhizobium* sp., *Azotobacter* sp., *Derxia* sp. respectively on 15th day such as *Rhizobium* sp., root length (9.56±0.88), shoot length (20.10±0.11) fresh weight (3.90±0.04) and dry weight (0.39±0.03). Whereas in *Azotobacter* sp, root length (9.06±0.08) shoot length (19.6±0.33), fresh weight (4.90±0.67) and dry weight (0.49±0.01) were lesser than the values promoted by *Dexria* sp. root length (8.06±0.88) shoot length (19.00±0.05), fresh weight (3.90±0.02), and dry weight (0.39±0.01) whereas in 3 ml of mixed culture (*Rhizobium* sp., *Azotobacter* sp., *Derxia* sp.) and isolated organism studies also the vegetative growth was highly promoted in mixed culture, root length (10.26±0.88), shoot length (20.76±0.88) fresh weight (6.80±0.01) dry weight (0.68±0.01). Such as *Rhizobium* sp., root lengths (9.60±0.05), shoot length (20.20±0.10) fresh weight (5.8±0.01) and dry weight (0.58±0.01). *Azotobacter* sp. vegetative growth values were root length (9.26±0.88) shoot length (20.13±0.88), fresh weight (5.80±0.65) and dry weight (0.58±0.03). *Derxia* sp. showed lesser vegetative growth promotion such as root length (8.20±0.057), shoot length (19.13±0.08), fresh weight (4.20±0.01) and dry weight (0.42±0.03). In 5 ml concentration of plant growth studies using mixed culture (*Rhizobium* sp., *Azotobacter* sp., *Derxia* sp.) and isolated organism studies also the vegetative growth was highly promoted in mixed culture, root length (9.63±0.33), shoot length (20.40±0.05) fresh weight (9.60±0.01) dry weight (0.96±0.04). The values vegetative growth parameters promote by *Rhizobium* sp. were root length (9.66±0.03) shoot length (20.86±0.08), fresh weight (7.40±0.01) and dry weight (0.74±0.01). Similarity vegetative growth promoted by *Azotobacter* sp. was root length (9.63±0.33), shoot length (7.20±0.05), fresh weight (7.20±0.01) and dry weight (0.72±0.04). *Derxia* sp. showed lesser vegetative growth promotion such as root length (8.36±0.06), shoot length (19.33±0.12), fresh weight (6.9±0.01) and dry weight (0.69±0.03) (Tien *et al.* 1979; Viviene *et al.* 2004; Egamberdieva, 2008; Zahir *et al.*2008; Selvakuma *et al.*, 2009).

Conclusion:

The bacterial strains were isolated from root nodules of Cowpea (*Vigna ungiculata)* and its rhizhosphere soil. The bacterial strains such as *Rhizobium* sp., *Azotobacter* sp. and *Derxia* sp. were identified using morphological and biochemical charecteristics. The identified bacterial strains such as *Rhizobium* sp., *Azotobacter* sp. and *Derxia* sp. were screened for IAA production. Then the plant growth promoting activity of three isolated bacterial strains such as *Rhizobium* sp., *Azotobacter* sp., *Derxia* sp. and mixed culture (*Rhizobium* sp., *Azotobacter* sp. and *Derxia* sp.) culture broth was tested at different concentrations (1,3 and 5ml) and at different days (5th

10^{th} and 15^{th}) intervals. The growth parameters such as root length, shoot length, fresh weight and dry weight have been enhanced by mixed culture than seeds treated with single cultures.

References:

Arshad, M. Frankenberger, W.T. (1998): Plant growth regulating substances in the rhizosphere: Microbial production and function. *Adv. Agron.* 62:46–51.

Cassan, F. Perriga, D. Sgroya, V. Masciarella, O. Pennab, C. Lunaan, V. (2009): AzospirillumbrasilenseAz39 and Bradyrhizobiumjaponicum E109, inoculated singly or in combination, promotes seed germination and early seedling growth in corn (*Zea mays* L.) and soybean (*Glycine max* L.). *Eur. J. Soil. Biol.* 45:28–35.

Egamberdieva, D. (2008): Plant growth promoting properties of rhizobacteria isolated from wheat and pea grown in loamy sand soil. Turk *J. Biol.* 32(1): 9–15.

Gaskins M' H, Albrecht S and Hubbell D H, (1985): Rhizosphere bacteria and their use to increase plant productivity: *A review. Agri. Eco. Env.* 21: 99 - 116.

Glick, B.R., Bashan, Y. (1997): Genetic manipulation of plant growth promoting bacteria to enhance biocontrol of phytopathogens. Biotechnol Adv. 15:353–378.

Kloepper, J.W., Ryu, C.M., Zhang, S. (2004): Induced systemic resistance and promotion of plant growth by Bacillus sp. Phytopathology 94:1259–1266.

Kloepper, J. W. and Schroth, M. N. (1989): Plant growth- promoting rhizobacteria on radishes. in: proc. Of the 4^{th} internat. Conf. on Plant pathogenic Bacter. Vol 2, Station de Pathologie et Phytobacteriologie, INRA, Angers, France. pp: 879-882

Selvakumar, G. Lenin, M. Thamizhiniyan, M. P. and Ravimycin, T. (2009): Response of biofertilizers on the growth and yield of blackgram (*vigna mungo L.*). *Recent Research in* Science and Technology, 1(4) pp: 169.175.

Tien, T.M., Gaskins, M.H. and Hubbell, D.H. (1979): Plant growth substances produced by *Azospirillum brasilense* and their effect on the growth of pearl millet *(Pennisetum americanum). Appl. Environ. Microbiol.* 37:1016-1024.

Viviene, N. Matiru and Felix, D. Dakora, (2004): Potential use of rhizobial bacteria as promoters of plant growth for increased yield in landraces of African cereal crops. *Af. J. of Biotech.* Vol. 3 (1), pp: 1-7.

Zahir, Z.A., Munir, A., Asghar, H.N., Shaharoona, B., Arshad, M. (2008): Effectiveness of rhizobacteria containing ACC deaminase for growth promotion of peas (*Pisum sativum*) under drought conditions. *J Micro. Biotech* 18(5): 958–963.

MULBERRY SERICULTURE- A PROMISING AND DEVELOPING AGRO BASED INDUSTRY IN MANIPUR

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Introduction:

Sericulture is a developing agro based industry in Manipur which is gaining attention in the recent years. Sericulture was first initiated in China. Sericulture involves the rearing of silkworm, harvesting of silk cocoon and processing to a fine silk thread. Silk is a natural fibre produced by silkworm and consists of a protein termed as sericin and fibroin. Silk is synthesized in the silk gland of the worm and it is secreted as a liquid silk through spinnerets and subsequently the liquid silk undergoes polymerization, thereby, forming a long silk fibre. Mulberry sericulture is most commonly practised in Manipur. It requires cultivation of mulberry, extensive production of silkworm eggs, rearing of silkworm, collection of cocoon and lastly, production of desirable silk thread. The small scale mulberry sericulture industries have been established in different locations namely, Andro, Leimaram, Khurkhul and Thongjao. After implementation of Manipur Sericulture Project Phase-I, about 5200 hectare of land was covered with mulberry plantation and about 12,000 farmers started practicing sericulture both as primary and secondary occupation.

The latest innovative silk worm rearing technologies have been introduced in Manipur in recent years. The production of Bivoltine and Multivoltine silkworm has been increased since last few years in Manipur due to greater demand.

Mulberry silkworm (*Bombyx mori)* is a monophagous insect which feeds only on Mulberry leaves. This insect has Uni, Bi and Multivoltine characters. It is under the family *Bombycidae* of the order *Lepidoptera.* The moth is native to China. Female moth is larger than the male moth. The moth cannot fly. Female secrete a hormone termed as bombykol for initiation of mating. Female can lay 300 to 500 eggs. Some important diseases of silkworm are muscardine caused by *Beauveria bassiana*, Grasserie caused by *Nuclear Polyhedrosis virus* (BmNPv) and also *Borrelina virus* (BmNPv), flacherie caused by *Streptococci, Staphylococci*,

Bacillus, *Serratia marcescens* alone or in combination with a virus and pebrine caused by *Nosema bombycis* (Chopade *et al.*, 2017).

Figure 1: Mulberry cultivation in Manipur

Figure 2: Mulberry silkworm (*Bombyx mori)*

Mulberry silkworm rearing technique:

Two distinct steps of rearing Mulberry silkworm are required namely;

1. Chawki or Early stage Silkworm rearing and
2. Adult or Late stage Silkworm rearing.

The two types rearing should be conducted separately at different stations or rearing house as per the latest rearing technology available for the individual farmer.

Chawki Silkworm Rearing:

Rearing of I, II & III instars is referred to as young age rearing or Chawki rearing. Young age worms are more resistant to high temperature and humidity and can grow healthier in such adverse conditions. Young age worms require tender and succulent mulberry leaves. Larvae achieved 400 times in body weight, 300 times in body size and 500 times in silk gland respectively.

Different methods of rearing Chawki silkworms:

The two methods employed commonly for Chawki rearing are paraffin paper rearing and box rearing. Box rearing is more popular than paraffin paper rearing. In the first method, paraffin paper is used as a bottom layer and as a cover for rearing beds to maintain optimum humidity. Feeding is restricted to 2 or 3 times a day in this method. The top paraffin paper must be removed at least 30 minutes prior to feeding for adequate aeration of the bed. In box rearing, boxes with lids or without lids of 10-15 cm deep boxes are used. Paraffin paper, wet foam rubber pad, etc are used as usual. Boxes with lids are placed in shelves while boxes without lids are piled one over the other, but in II instar, a space of 2-3 cm between boxes is provided for ventilation. The boxes with lids are not covered during III instar. However, prior to moulting, the paraffin paper, wet foam pad and the lids should be removed to keep the bed dry.

Chawki rearing of mulberry silkworm comprises of the following steps:

1. **Incubation**: Eggs are incubated at optimum temperature of 24-26^{o}C and relative humidity of 70-80% under 16 hours in light and 6 hours in dark conditions to ensure uniform hatching.
2. **Black Boxing:** The eggs which are incubated under optimum conditions reached pin head or head pigmentation stage by 7/8th day i.e. 48 hours before hatching. On next day, i.e., 24 hours before hatching, the eggs turn bluish to brownish colour which is referred to as "blue egg or body pigmentation stage". The eggs which are kept in darkness during pin head stage are referred to as "black boxing of egg." Darkness arrests the hatching of the fully developed eggs but facilities lagging of embryo to enhance faster development. Eggs after black boxing are stimulated to hatch by exposing them to sunlight or any bright light stimulus.
3. **Brushing**: Brushing involves separation of newly hatched larvae gently and carefully from empty egg shells or egg sheets and transferring them to the rearing sheets. After black boxing is opened and exposed to light, good hatching is obtained in the next morning

which is ideal time for brushing also. Fine camel hair brush is generally preferred for brushing.

Environment conditions required for Chawki Silkworm Rearing:

The ecological factor namely temperature, relative humidity and light have significant influence on the growth of larvae. The standard temperature and humidity recommended are as follows.

Instar	Temperature	Relative Humidity (RH)%
I	28.29	90
II	27.28	85
III	26.27	80

During moulting, low humidity of 70% RH is preferable and is maintained by using paraffin papers and wet form pads for covering the rearing bed respectively. Dim light of 15 to 30 lux influences uniform distribution of larvae in rearing bed as the worms are more crowded in dark. Photoperiod during early instar influences the type of eggs produced, i.e., hibernating or non-hibernating. A photoperiod of 16 hours light 8 hours dark is ideal for rearing young age instar. The circulation of fresh air in rearing room is important for silkworm rearing.

Collection of mulberry leaves:

Mulberry leaves for Chawki rearing should be soft, succulent and high in water content, protein, carbohydrates etc. The largest glossy leaf method is preferable for Chawki rearing so as to facilitate plucking of correct leaves. The largest glossy leaf is the one which is light green and glossy, being the largest among the first few leaves. From glossy leaf to 5 or 6 leaves further below are desirable for I instar, another 4 or 5 leaves further below are desirable for II instar and further down up to all tender leaves are desirable for III instar.

Preparation of bed:

Maintenance of optimum bed area is important to attain full larvae growth, good survival and successful cocoon formation. For Multivoltine x Bivoltine hybrid, 360 feet and for Bivoltine x Bivoltine 480 feet bed is recommended. 2/100 disease free layings are found to be optimum by the end of V instar. Rearing bed area is required to be increased on a daily basis so as to match the growth of silkworms.

The space requirement for larvae during Chawki rearing is given below:

Uni/ Bivoltine			**Multivoltine**	
Instar	Beginning	End	Beginning	End
I	0.2	0.8	0.2	0.5
II	1.0	2.0	0.5	1.5
II	2.0	4.5	1.5	3.0

Bed cleaning:

Accumulation of leftover leaves and litter in rearing bed leads to increase in humidity and temperature and hence, multiplication of various pathogens resulting in diseases. Therefore, regular cleaning of bed is necessary. Cotton or nylon nets should be used for bed cleaning since the young age worms are delicate. The cleaning net should be applied on the bed and the mulberry leaves are to be given above the net so the worms can crawl through the meshes and eventually feed on the leaves on the provided on the net. At the next feeding, the net along with the worms should be transferred to another bed and fresh mulberry leaves are to be given. The faecal matter and left over feed are to be removed and the bed is to be cleaned.

Bed cleaning should be done once in case of I instar and twice in case of II instar viz., once after resumption and once before settling for second moulting and thrice during III instar on resumption of feeding, second during the middle and third prior to III moult. The mesh size of nets used for I & II instars is usually smaller measuring 2 mm^2 and for III instars 10 mm^2 respectively.

Identification of moulting larvae:

Moulting larvae are identified by feature such as raised head, pointed mouth portion when the larvae are going for moult.

Handling of silk worm during moult:

Silkworms usually stop eating just prior to moult. The rearing bed should be thinned out for drying and layer of lime powder should be sprinkled. Comparatively low humidity (70% RH) is desirable during moulting. Worms in moult should not be disturbed and agitated and there should be good ventilation. During III instar, paraffin paper should be covered only on the top but not at the base and use of foam pads must also be dispensed.

Adult silkworm rearing:

The IV and V instars silkworms are late age larvae of silkworms. Late age worms require slightly lower temperature and humidity. During this period the silk body increases in size by 29 times, in weight by 25 times, and silk gland weight by 200 times.

Methods of rearing adult stage silkworm:

There are three methods commonly known for late age rearing.

1**. Shelf rearing**: Silkworms are reared on rearing trays and should be arranged one over the other in tiers on rearing stands. Each rearing stand can accommodate up to 10 trays. Cleaning should be more frequent in this method and the amount of labour requirement is greater. However, it accommodates more silkworms in a limited area.

2. **Floor rearing:** Silkworms are reared on fixed rearing seats arranged in two or three tiers. The rearing seats measures 5-7 meter in length and 1-1.5 m in width. The space between tiers should be 0.6 to 0.8 meter. Bed cleaning is similar as for shelf rearing, twice during IV instar and thrice during V instar. The floor rearing requires less labour but maximum space.

3**. Shoot rearing**: Silkworms are reared on big branches in on or two tiers. The big shoots harvested from field are fed to the worms. It is similar to floor rearing. The rearing seats are 1 meter in width and can be of any length. In case of single tier, rearing seat 20cm above ground and in case of two tier, 1 meter gap is maintained between the tiers. In this method, shoots should be supplied for every feed and the larvae are to be kept moving upward and are distributed in three dimensions. It provides better aeration of rearing beds. Bed cleaning is done once each in IV instar and V instar. Ropes of appropriate length are spread parallel to each other on the bed. Fresh feed should be supplied and after two to three feeds, all the worms will crawl to new branches. Beds should be then cleaned thoroughly and a fresh bed should be subsequently prepared. It requires less time and is not labour intensive.

The different aspects of old age rearing are as follows:

Environmental conditions:

The ideal standard rearing temperature and humidity during late age rearing are as follows:

Instar	Temperature	Relative humidity (%)
IV	24-25	75
V	23-24	70

Temperature above 36^0C affects survival and pupation. Comparatively low humidity is desirable during moulting. Rearing rooms should have good ventilation. It is essential to ventilate the rearing rooms from IV instar by removing paraffin paper cover at least for one hour before each fresh feed of mulberry leaves. CO_2 exceeding 1% in a rearing room is bad for silkworms. Air current of 1.0m/s during V instar considerably reduces larval mortality. Dim light of 15 to 20 lux is desirable and strong light or complete darkness should be avoided. A photoperiod of 16 hours light and 8 hours dark are also desirable during late age rearing.

Feeding:

Mulberry leaves should be plucked in morning. Care should be taken to avoid plucking of too tender or too mature and yellow leaves. Clipping off the terminal buds in row system, a week prior to shoot harvest is desirable for IV and V instars. Quantative requirement of feed in late age usually forms the bulk of the total larval feed.

The feed requirement for 100 disease free layings is as follows:

Leaf quantity in kg		
Instar	**Uni/Bivoltine**	**Multivoltine**
IV	80-90	35-90
V	450-475	335-325
Total	530-565	335-375
Grand total from I to V instars	550-600	350-400

Leaves harvest should be usually done by individual leaf plucking or shoot harvest. In leaf plucking, whole leaf is fed without cutting. In case of shoot harvest, leaves should be cut in to convenient length so as to accommodate in rearing trays. Four feeds per day should be practice in late age rearing which can be reduced in rainy season while increased in summer without changing the total quantum of feed. The feeding time usually is around 5.00 am, 11 am and 1.00 am.

Bed spacing:

Adequate bed spacing is essential for the success of silkworm rearing. The spacing should be increased daily in proportion to the growth of silkworm. The rearing bed spacing recommended for IV and V instars are as follows.

Space in mm/larvae				
Instar	**Uni/Bivoltine**		**Multivoltine**	
	Beginning	End	Beginning	End
IV	5.0	10.0	3.0	9.0
V	10.0	20.0	9.0	18.0

Over crowded rearing should be avoided as it will leads to insufficient consumption of feed, poor growth, susceptibility to diseases and low cocoon yield of inferior quality.

Bed cleaning:

Bed cleaning should be done every day with nylon or cotton nets of appropriate mesh size (20mm or 2cmX 2cm) during IV and V instars. In Chawki rearing, the net should be fixed over the rearing tray just prior to cleaning and feed should be given above the net. Before next feeding, the net along with larvae and leaves should be transferred to another tray. The faeces and left over leaves are to be removed regularly. Paddy husk or charred husk can also used for bed cleaning in late age rearing which are to be spread in a thin layer over the bed prior to feeding. The worms will eventually crawl through this layer and subsequently start feeding on fresh leaves. The worms and the leaves are to be removed and transferred to a fresh tray and the litter and old leaves are to be put in a manure pit.

Points to be noted:

1. The optimum environmental conditions differ for early and late stage rearing. Environmental conditions should be adjusted according to growth stages of the silkworms.
2. Tender and succulent leaves should be fed to early stage silkworm
3. Too tender and young leaves and too mature and yellow leaves should not be fed in late stage rearing.
4. Bed spacing should be appropriate according to growth stages.
5. Bed should be cleaned thoroughly.
6. Lime powder should be dusted after every moulting.
7. Silkworm should be periodically suspected for diseases.
8. Appropriate management practices should be taken up in case of disease incidence in silkworm.
9. Maintenance of proper hygiene to avoid diseases.
10. Disease free mulberry leaves should be preferred for feeding.

Future Prospects of mulberry sericulture in Manipur:

Mulberry silkworm rearing requires technical skill. In the last few years, cooperative rearing centres have been organized in Manipur which provides ideal rearing houses with necessary equipment and the silk worm are reared by technical experts. Training programmes are being organised by government, private companies and NGOs. Scientific rearing in cooperative rearing centres ensures that the worms are vigorous and healthy and produce good quantity of silk. Young age worms raised in collective Chawki centres or cooperative rearing centres. These centres distribute III instar silkworm larvae, different types of rearing beds or tray. They even provides bed that can be rolled as many as 5 to 6 and such rolls are arranged in wooden trays for transportation. The worms are usually transported from these centres during cooler hours of the day either in morning or late in the evening. The benefits of mulberry silkworm rearing is recognized to a great extend since the recent years and many small scale farmers had opted for this agro based industry as a source of their livelihood.

Figure 3: Small scale rearing of mulberry silkworm in Manipur

Conclusion:

Mulberry silkworm rearing is getting popularised in Manipur in recent years. It is being adopted as one of small scale industries in many parts of Manipur. It not only generates generous amount of income but also provide employment. It is now being considered as a source of livelihood. Mulberry plantations are increased since last few years for the purpose of Mulberry silkworm rearing. As the climate of the state favours Bivoltine mulberry silkworm rearing, the

farmers have grasped this opportunity to turn it into full time employment and produce good quantity of silk. Moreover, the farmers are benefited by latest mulberry silkworm rearing techniques provided by the several experts and NGO training programmes. The farmers are also encouraged by the introduction of simple rearing technique of silkworm. Therefore, mulberry silkworm rearing is gaining lot of attention due to its immense potential.

References:

Chopade P, Raghavendra CG, Kumar MS and Bhaskar RN (2017): Assessment of diseases in *Bombyx mori* silkworm –A survey. Global Transitions Proceedings 2(1)::133-136.

Datta, R.K. (1984): Improvement of silkworm Races (*Bombyx mori L.):* Sericologia 34(3):: 393-415.

Datta, R.K. (2000): Evaluation of new productive Bivoltine hybrid CSR_3. Sericologia 40:407-416.

Datta, R.K., Suresh Kumar, N., Basavaraja, H.K., Kishor Kumar, C.M. and Reddy, N. (2001): CSR_{18} CSR_{19} A robust Bivoltine hybrid for all season rearing. Indian silk 39 (12):: 5-7.

Dwarakinath, R. (1994): Guide to Sericulture Extension. Central Silk Board Publication, Bangalore. 115p.

ESCAP Publication (1993): Principal and Techniques of Silkworm Breeding. 114p. FAO Agriculture services bulletin Sericulture: Manuals.

Krishnaswamy, S.A (1993): Practical guide to mulberry silk cocoon production in tropics. CSB Publication, Bangalore India 101p.

Mukherjee, N.G. (1912): Handbook of Sericulture, Bengal Secretariat Book depot Calcutta, India.

MOLECULAR GENETIC TECHNOLOGIES ISOLATED EXPRESSED VALUABLE PROTEIN PRODUCTS

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Abstract:

At the heart of most molecular genetic technologies is the gene. A gene must be isolated and well characterized before it can be used in genetic manipulations. One method of isolating and amplifying a DNA of interest is to clone the gene by inserting it into a DNA molecule that serves as a vehicle or vector. When these two DNAs of different origin are combined, the result is a recombinant DNA molecule. The molecule is moved into a host—*Escherichia coli*, for example—where it can be reproduced. When the cells divide, each cell, or clone, in the colony contains one or more identical copies of the recombinant DNA molecule. K N Kao (1990), G Melchers (1974).Thus, the DNA contained within the recombinant molecule is cloned. Gene cloning has many uses. To name just a few, DNA can be amplified in host cells to obtain many copies for further study, a gene can be expressed to obtain a valuable protein product, and a gene and its expression can be studied in a living cell. In this chapter the basic methods of isolating and characterizing DNA are described, with emphasis on the preparation and use of recombinant DNA.

Keywords: Moleculargenetics, technologies, protein product, expressed, isolataion

Introduction:

All phage vectors used as cloning vectors have been disarmed for safety and can function only in special laboratory conditions. Recombinant DNA containing the viral DNA and the DNA of interest are packaged into viral particles in the test tube (Jeff Schell, 1987; Rosset, 1989). Host bacterial cells are infected with recombinant phage DNA, the DNA replicates within the host cells, and progeny phage are produced when the host cell undergoes lysis. Plaques then become visible on plates where cells in the colony have lysed. The DNA of lambda-type phage can accommodate only an additional 3 kb, or 5%, of its genome, for a total size of 52 kb. DNA that is too large or too small cannot be packaged into the head or the viral particle. Fortunately, a

significant portion of the viral genome can be deleted without adversely affecting packaging and infection of *E. coli* host cells (Fernanda *et al.*, 2000; Ramezan, 2011).

Table 1.1: Examples of some Restriction Endonucleages and their Properties

			Characteristics	
Restriction Endonuclea	**Source (Bacterial Species)**	**Target site (Cuts at**	**Recognizes (No. Base Pairs)**	**Product**
EcoRI	*Escherichia coli* Rl 3	↓ G-A-A-T-T-C C-T-T-A-A-G	6	4-base-long sticky ends
Hha I	*Haemophilus baemolytkus*	↓ G-C-G-C C-G-C-G ↑	4	2-base-long sticky ends
Sma I	*Serratia marcescens*	↓ C-C-C-G-G-G G-G-G-C-C-C	6	Blunt ends
Hae III	*Haemophilus aegyplius*	↓ G-G-C-C C-C-G-G	4	Blunt ends

Cutting and joining DNA:

Two major categories of enzymes are important tools in the isolation of DNA and the preparation of recombinant DNA (in which two DNA molecules are combined). A specific DNA or gene is removed from the DNA by cutting the sugar-phosphate backbone, and the DNA from two different sources are mixed and recombined. Recombinant DNA molecules cannot be easily generated without two types of enzymes: restriction endonucleases that act as scissors to cut DNA at specific sites and DNA ligase that is the glue that joins two DNA molecules in the test tube. Restriction endonucleases cut both strands of the DNA sugar-phosphate backbone. Restriction endonucleases recognize specific sequences within the DNA molecule (Figure 1.1). The recognized sequences are usually four to six base pairs and are palindromic (Table 1.1): the sequence of both DNA strands are the same when read in the same direction, the 5' to 3' or 3' to 5' direction.

5'GCCAATTGGC3’

3'CGGTTAACCG5'

Each restriction enzyme recognizes a specific sequence and cuts at a particular place within that sequence. The enzyme cuts the double strand of DNA by breaking covalent bonds between the phosphate of one deoxyribonucleotide and the sugar of an adjacent deoxvribonucleotide. Restriction endonucleases are found primarily in bacteria, where they cut, or fragment, foreign DNA of bacteriophage before the invading DNA can replicate within the host bacterial cell to produce new phage that would ultimately destroy the host. The bacterial cells are resistant because their DNA is chemically modified, primarily by the addition of methyl groups, to mask most of the restriction endonuclease recognition sites so that these will not be cut (Handley *et al.*, 1986; Drew, 1993; Vasil, 2002; Tudses,2014, 2015).

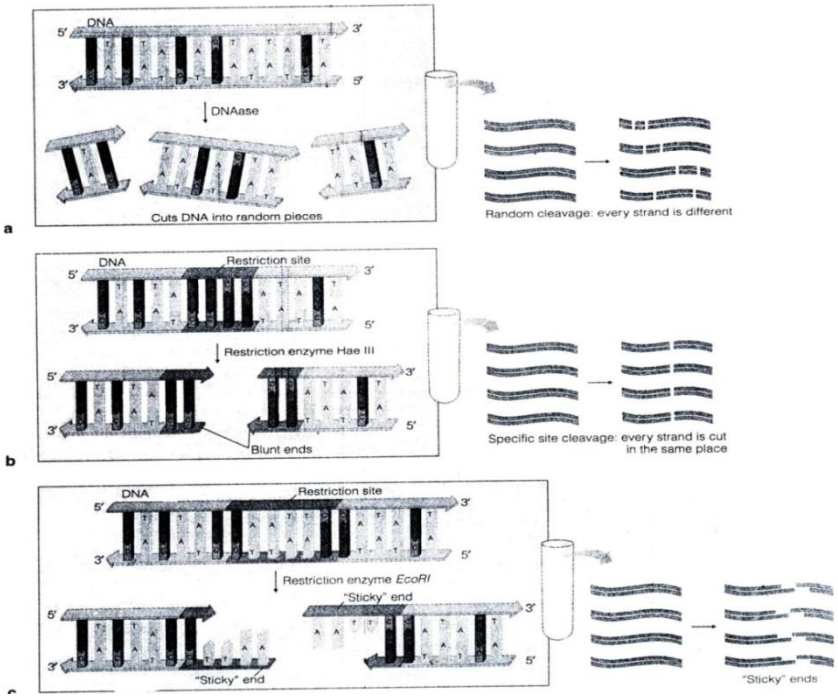

Figure 1.1: Cutting double-stranded DNA. (a) The enzyme DNase cuts DNA at random sites. Each DNA molecule *in* the test tube can be cut at different sites, (b) and (c) A specific restriction enzyme cuts each DNA molecule at the same sequence site. Some restriction enzymes make a blunt cut (for example, *Haelll)* and others make a staggered cut (for example, *EcoRI)*

Restriction enzymes are named for the organisms from which they are isolated. For example, *EcoRI* is isolated from *E. coli* RY13: *Eco* comes from the first letter of the genus name and the first two letters of the species name; R is for the strain type and 1 is for the first enzyme of that type. Thus, *Bam HI* is isolated from *Bacillus amyloliqucfaciens* strain H, and *Sau3A* is isolated from *Staphylococcus aureus* strain 3A. Restriction enzyme cleavage of a sugar—phos-

phate backbone can produce a double-strand DNA fragment with blunt or staggered ends. When both strands of the molecule are cut at the same position, the ends are flush and no nucleotides are left unpaired; this is a blunt end. When each strand of the molecule is cut at a different position so that one strand (the 5' or the 3') overhangs by several nucleotides, these single-strand ends can spontaneously base pair with each other—that is, they are sticky, or cohesive (Szarka *et al.,* 2002; Xhu *et al.,* 2003).

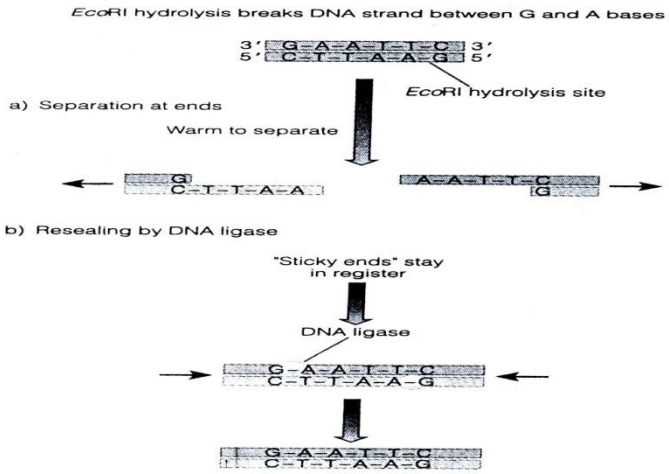

Figure 1.2: The ligation of two different pieces of DNA. The overlapping, cohesive ends of each DNA anneal and DNA ligase form phosphodiester bonds

Separating restriction fragments and visualizing DNA:

Restriction enzyme digestions and other manipulations of DNA enable the results to be directly visualized. Agarose gel electrophoresis is a technique for separating DNA fragments by size and visualizing them after staining (Figure 1.3). Gelatinous agarose is a mixture of a powder of purified agar (isolated from seaweed) and buffer, which is boiled and poured into a mold where the agarose (generally 0.7 to 2.0%) gels and solidifies into a slab (Grosser, 2011; Tiwari *et al.,* 2010, 2011; Barbara *et al.,* 2015). A toothed comb forms wells in the molten agarose, and samples are loaded into the wells after the agarose solidifies. The agarose slab is submerged in a buffer solution and an electric current is applied to electrodes at opposite ends of the slab to establish an electric field in the gel and the buffer. Because the sugar-phosphate backbone is negatively charged, the DNA fragments migrate toward the positive electrode. Pores between the agarose molecules act like a sieve that separates molecules by size. Larger molecules move more slowly than smaller molecules. Increasing the percentage of agarose produces smaller pores; these increase the resolution of smaller fragments by impeding all but the smaller molecules (Ahloowalia, 1991; Amberger *et al.,* 1992).

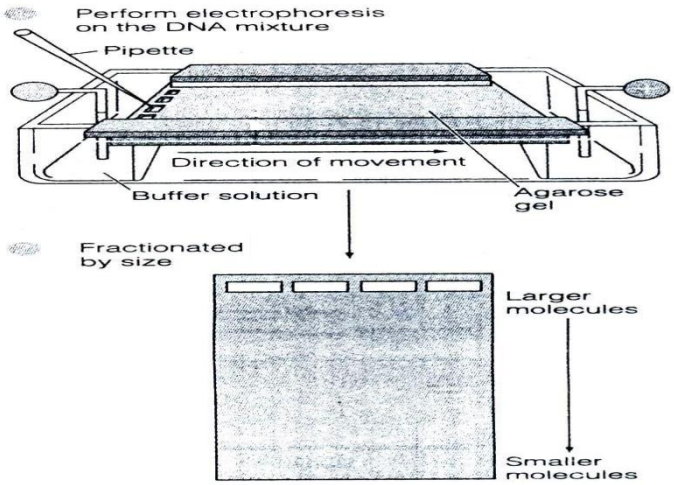

Figure 1.3: Agarose gel elactrophoresis is used to separate DNA (and RNA) molecules according to size. The negatively charged nucleic acid moves toward the positive electrode. Larger molecules move more slowely than smaller molecules

Lowering the percentage (amount) of agarose produces larger pores; these increase the resolution of larger fragments by allowing greater separation among them Li the gel. Because DNA by itself is not visible in the gel, ethidium bromide is usually added to make the DNA bands visible. Ethidium bromide molecules intercalate between the bases causing the DNA to fluoresce orange when the gel is illuminated with ultraviolet light. Other dyes (nonhazardous) that stain DNA include methylene blue; however, most research laboratories use ethidium bromide because it is very sensitive and allows the detection of very small amounts of DNA. The lengths of the DNA fragments can be determined by comparing their position in the gel to reference DNAs of known lengths also in the gel. A DNA fragment migrates a distance that is inversely proportional to the logarithm of the fragment length in base pairs over a limited range in the gel (Beversdrof *et al.*, 1977; Bellincampi *et al.*, 1987). Thus, agarose gel electrophoresis allows the restriction fragment lengths to be determined.

DNA cloning:

There are several steps involved in cloning a gene. The specific methodology used in each step may vary depending on the type of DNA used, the host cell type (for example, bacterial versus plant), and the ultimate goal of DNA cloning. For example, the type of vector used for cloning will depend on whether the cloned DNA will remain in the vector or be inserted into the chromosome of the host cell (by recombination). A scientist also may want to retrieve a product from the gene (for example, human insulin or human growth hormone) and will use a vector that allows-the gene to be expressed in the host cell (Binding, 1974; Buttenko, 1980).

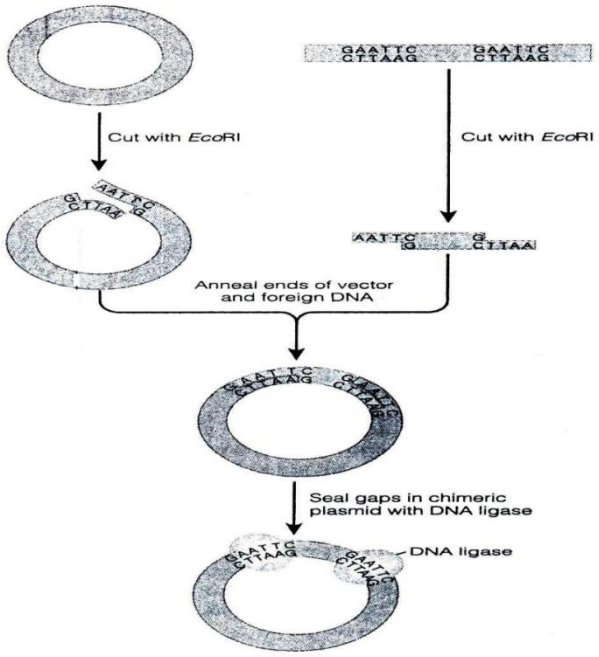

Figure 1.4: Ligation of a DNA fragment into a vector using the restriction endonuclease *EcoRI* and DNA ligase

The vector must then have the important promoter sequences for transcription to take place and other sequences for translation. The steps in gene cloning include.

- Isolation of DNA
- Ligatmg the DNA into a vector (Figure 1.4)
- Transformation of a host cell with the recombinant DNA (vector DNA with DNA insert) (Figure 1.5)
- Selection of host cells harboring the recombinant DNA
- Screening of cells for those harboring the recombinant DNA or producing the appropriate protein product

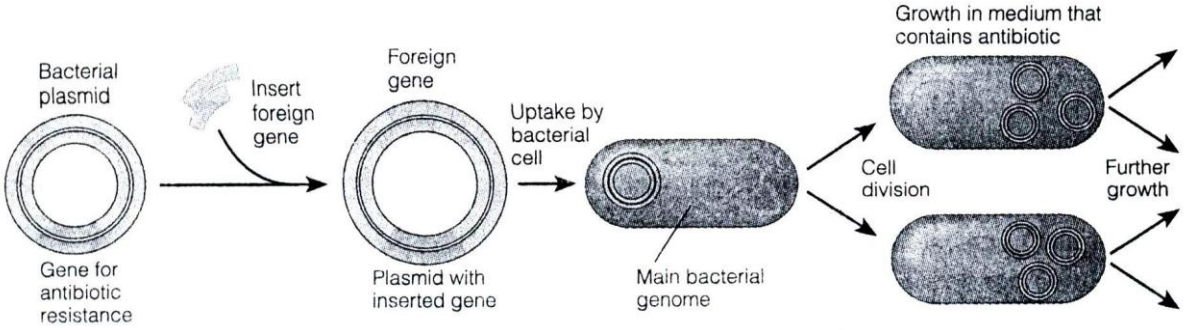

Figure 1.5: The transformation of bacteria and the selection of cells harboring the recombinant vector

Cloning vectors:

As discussed earlier, restriction endonucleases and DNA ligase are important enzymes in the production of recombinant DNA. Often a foreign DNA is introduced into a host cell by inserting it into a cloning vehicle or vector that transports it into the host cell. Many of these vectors replicate independently in a host cell. A cloning vector must

- Have an origin of replication so that the DNA can be replicated within a host cell
- Be small enough to be isolated without undergoing degradation during purification
- Have several unique restriction sites for cloning a DNA fragment so that the vector will be cut only once and several restriction sites for insertion will be available
- Have selectable markers for determining whether the cloning vehicle has been transferred into cells and to indicate whether the foreign DNA has been inserted into the vector
- Bacterial Vectors

Bacterial vectors:

The greatest variety of cloning vectors has been developed for *E. colt* because of the major role they have played in recombinant DNA experiments since the 1970s. Other cloning vectors are available for bacteria, such as *bacillus subtilis,* as well as for yeast, fungi, animals, and plants.

Plasmids:

Bacteria harbor plasmids: circular double strands of DNA that is extrachromosomal; that is, they are not part of the bacterial chromosome. Often multiple copies of plasmids are present in the cell. Plasmids have diverse functions. Some encode substances for antibiotic resistance and bacteriocins agents that kill or inhibit similar bacterial straits or species. Others perform physiological functions, such as pigment production, degradation of compounds, and dinitrogen fixation. Toxin-producing and virulence plasmids encode endotoxins and hemolysins, whereas some plasmids confer resistance to metals such as mercury, cadmium, nickel, and zinc. Because plasmids are relatively small and easy to manipulate, they have been engineered as cloning vectors with unique restriction sites for insertion of foreign DNA fragments up to approximately 10 kilobase s long (1 kilobase, or kb, equals 10(10 nucleotides). Christianson (1983), Cocking EC (1960).When transported into bacterial cells, recombinant plasmid vectors are readily replicated, often in great numbers, or high copy number.

An early vector, pSClOl, used by Cohen, Boyen and their colleagues replicated to yield only one or two copies in each cell (low copy number). Col El, a high copy plasmid developed in 1974, produced several thousand copies in one cell (Duke, 1981; Ghazi *et al.,* 1986; Gaj *et al*.,

2001). Col El was further modified so that several unique restriction sites were available for the insertion of DNA. One of these modified plasmids was pBR322, constructed in Boyer's laboratory (Figure 1.6). The name pBR322 is derived as follows: p identifies the molecule as a plasmid; BR identifies the original constructors of the vector (Bolivar and Rodriquez); 322 is the identification number of the specific plasmid (other examples are pBR325, pBR327, and pBR328).

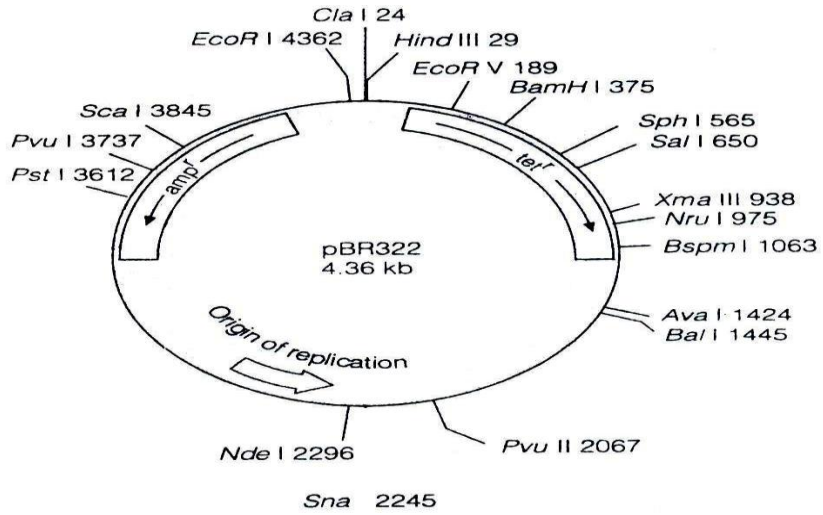

Figure 1.6: Restriction amp of pBR322 showing unique restriction sites, the ampicillin and tetracycline resistance genes, and the origin of replication

pBR322 is derived from three naturally occurring plasmids: the ampicillin-resistance gene from the plasmid Rl, the tetracycline resistance gene from pSClOl, and the replication region from pMBl. Although' many engineered plasmid vectors now have similar and even more powerful features, until recently pBR322 was one of the most commonly used plasmids for several reasons:

1. The molecule is small, having only 4363 base pairs, and can be isolated easily. Consequently, this vector can accommodate DNA of up to 5 to 10 kb.
2. pBR322 has several unique restriction sites where the plasmid can be linearized and opened for inserting a DNA fragment.
3. The genes encoding resistance to ampicillin *(amp')* and tetracycline *(tef)* are used for plasmid and DNA insert selection. The DNA fragment of interest can be inserted into one of the antibiotic resistance genes, inactivating that gene. The other antibiotic gene remains active and can be used to select for bacteria carrying the plasmid. Therefore, if a foreign DNA is inserted into the tetracycline gene, the bacteria containing such a plasmid would be ampicillin resistant but tetracycline sensitive *(amp'tef)* and will die if treated

with tetracycline. This process is referred to as insertional inactivation of a selectable marker. Hansen (1999), Henry *et al* (1998). A gene that gives a characteristic phenotype (physical characteristic) such as resistance to an antibiotic is inactivated, thus making the cells sensitive. One can easily screen cells for antibiotic resistance.

Insertional inactivation is a powerful way to determine whether the vector contains a DNA insert. The processes of restriction endonuclease digestion, ligation, and transformation are inefficient processes—that is, not all molecules are cut, ligated, or transferred to host cells. Consequentiy, in a population of bacterial cells that have been transformed, some cells have (1) received a recombinant vector, (2) received a vector not containing a DNA insert (vector only), or (3) not received a vector. A screening process allows the detection of cells that receive a recombinant DNA (vector with DNA insert). This process selectively kills cells that contain a recombinant DNA by exposure to an antibiotic because the DNA inserted into the vector inactivated an antibiotic resistance gene (Figure 1.7). Selectively .killing cells with antibiotics and identifying host cells harboring recombinant DNA involves the following:

1. After transformation of host cells with recombinant molecules produced in the laboratory, colonies must first be plated from the original, master plates to identical replica plates. Different antibiotics are added to the medium to identify (that is, select) cells with recombinant molecules.
2. To maintain the original bacterial colonies so that recombinant colonies from the master plates can later be further analyzed, exact copies are made by replica plating**.** The technique is conducted as follows:

a. After colonies of bacterial cells have grown in a medium such as agar, a sterile velveteen pad is pressed against the master plate (the dish holding the colonies). Cells from the colonies adhere to the velveteen (other transfer media can be used, such as nylon filter paper). The location of the cells on the velveteen is a mirror image of the locations of the original colonies on the master plate.

b. The velveteen pad is then pressed against media in a second and third plate, transferring cells to them. The locations of these cells will now be identical to the original colonies on the master plate.

c. Recombinant colonies are identified (and therefore selected) by the addition of different antibiotics to the replica plates and a comparison with the colony growth on the different replica plates.

3. When cells are placed onto ampicillin-containing agar medium, only cells with pBR322

will be able to survive; untransformed cells will not survive. To distinguish recombinant plasmids, bacterial colonies are replica plated onto a medium with tetracycline and ampicillin. Cells that survive do not have the DNA insert in pBR322 vectors, and cells that die have the DNA insert in pBR322. Thus, these cells are ampicillin resistant—tetracycline resistant ($amp^r tet^r$) and ampicillin resistant—tetracycline sensitive ($amp^r tet^s$), respectively.

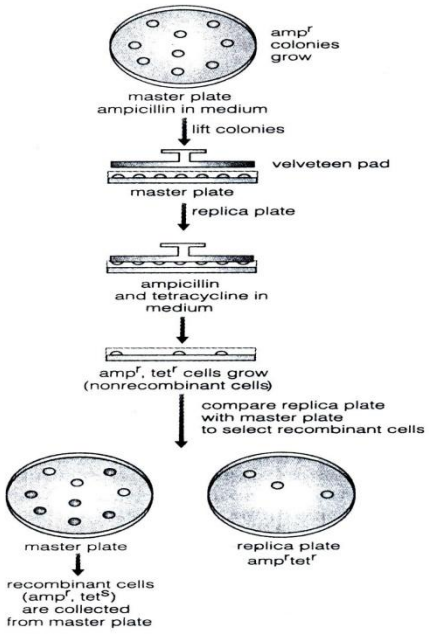

Figure 1.7: Selecting for recombinant cells after transformation

4. The master plate is examined to select those colonies that contain recombinant plasmids**.**

Today, pBR322 is seldom used because of its limitations—screening for cloned inserts is time consuming, and a limited number of restriction sites for cloning are available. However, many plasmid vectors in use are derived from this early vector. More commonly used are small plasmids containing selection functions that allow a more direct screening approach, such as the pUC plasmids developed in 1982. These small vectors (less than 4 kb) contain a poly cloning site made up of multiple restriction sites, where foreign DNA can be inserted. A series of pUC vectors containing the ampicillin resistance gene for plasmid selection has been constructed (for example, pUC8, pUC9, pUC18, pUC19). The multiple cloning sites differ in the type of restriction sites and their orientation (Figure 1.8). Selectable markers for insert selection are not limited to antibiotic resistance genes; the genes may encode enzymes that catalyze metabolic reactions. A chemical indicator aids in the identification of recombinant plasmids

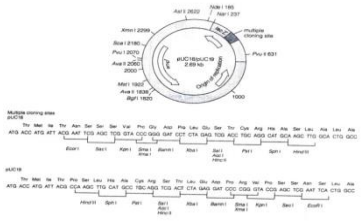

Figure 1.8: Restriction map of pUC18/pUC19 showing the ampicillin resistance gene, the origin of replication, and the multiple cloning sites within the *lacZ* gene. The orientation is reversed in pUC18 and pUC19 with the *EcoRI* site and the *Hindlll* site of the multiple cloning site immediately downstream from the lac promoter in pUC18 and pUC19, respectively

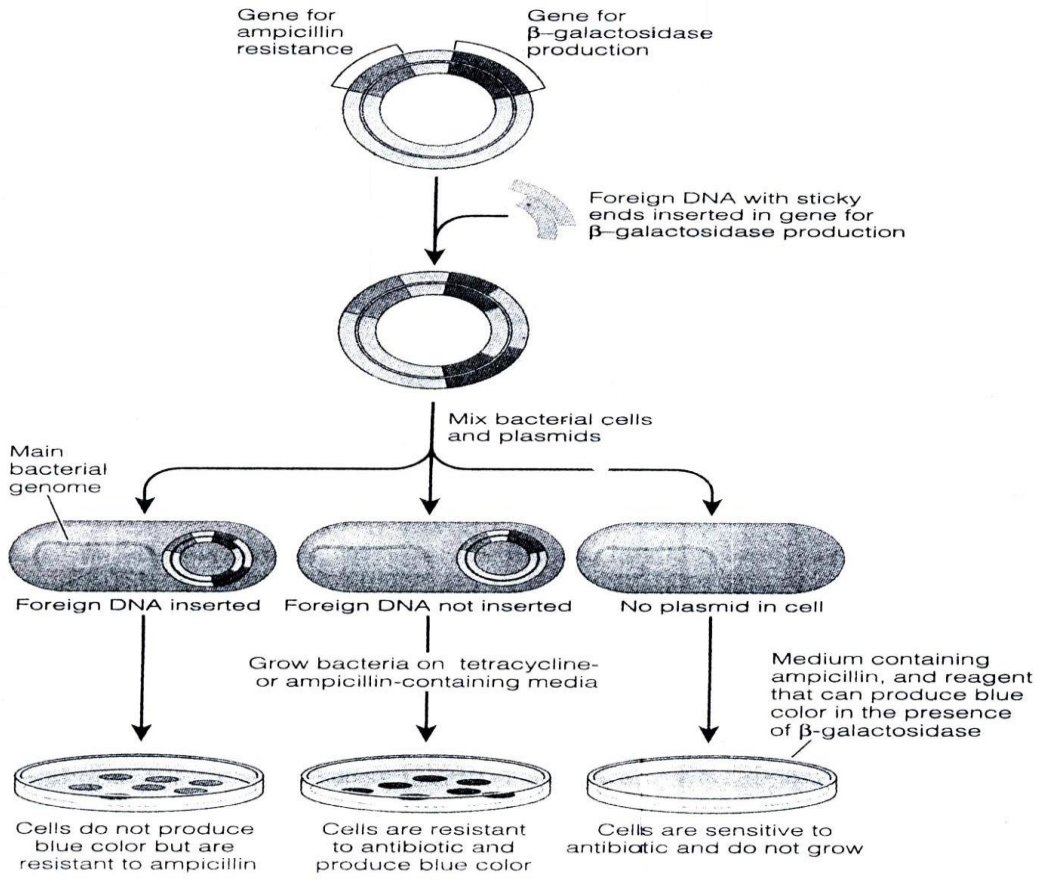

Figure 1.9: The selection of bacterial colonies containing a recombinant vector (pUC vector + DNA insert) by the selection of white colonies. The galactosidase gene is interrupted by a forein DNA insert

One type of selection, called α-complementation, allows the detection of DNA inserts in pUC plasmids. These vectors contain a portion of the *lacZ* gene (called *lac Z')* that encodes the first 146 amino acids for β-galactosidase. The polycloning site resides in this coding region. If the *lac Z* region is not interrupted by inserted DNA, the amino terminal portion of the β-

galactosidase polypeptide (lacZ) is synthesized. An E. *coli* deletion mutant called *lacZ'M15* harbors a chromosomal mutant of *lacZ* that encodes only the carboxyl end of the β-galactosidase. Both the plasmid and chromosomal *lacZ* fragments encode nonfunctional proteins. However, by α-complementation the two partial proteins can associate and form a functional β-galactosidase. In cells, β-galactosidase normally hydrolyzes the sugar lactose into glucose and galactose. The α-complementation is indicated by the presence of blue colonies on special media. The blue indicates that the plasmid-encoded *lacZ'* has combined with the partial lacZ from the complementary fragment of the chromosomal *lacT* gene residing on the chromosome of *E. coli,* generating an enzymatically active β-galactosidase that turns a chromogenic agent blue. The chromogenic lactose analog, X-gal, or 5-bromo-4-chloro-3-indoyl-β-D-galactopyranoside, when added to the medium is broken down by β-galactosidase and produces a blue color. When the plasmid *lacZ'* gene fragment is interrupted by the insertion of a foreign DNA fragment, colonies appear white on media (Figure 1.9).

Variation of Bacteriophage:

A virus that infects a bacterium is called a bacteriophage. Viral DNA can be engineered for use as a cloning vector; the first to be used was from a lambda bacteriophage, in 1974. Today many variations of lambda exist. Lambda phage vectors are derived from the 50-kb wild-type double-strand genome that has single-strand complementary ends of 12 nucleotides (cohesive termini, or *cos)* that can base pair. The *cos* ends (important for the lytic pathways) base pair, forming a circular DNA molecule once the phage DNA is inside the host cell. DNA replication then occurs from the circular molecules, producing linear lambda DNA made up of several 50-kb phage DNA end on end. In the lytic pathway (cycle), the host cell lyses after phage reproduction, releasing progeny virus (Figure 1.10). Lytic phages are used to clone and amplify a DNA of interest. The resulting plaques—cleared areas on the medium where host cells have lysed—contain millions of; recombinant phage particles that can be isolated (Figure 1.11). In the lysogenic pathway, the bacteriophage genome is integrated into the host cell, no cell lysis occurs, and the phage genome replicates along with; the host genome. For example, in gene therapy, animal viruses are used to integrate a cloned therapeutic gene into the genome.

Removal of one-third of nonessential DNA from the central portion of the phage allows a DNA insert of up to about 20 kb to be ligated into the phage DNA. The deletion contains most of the DNA necessary for integration and excision from the *E. coli* genome and is not necessary for use as a cloning vector. Vectors that have a segment of nonessential DNA removed, making room for foreign DNA, are called replacement vectors. Another type of phage vector, called an insertion vector, has one restriction site for the insertion of DNA of 5 to 10 kb.

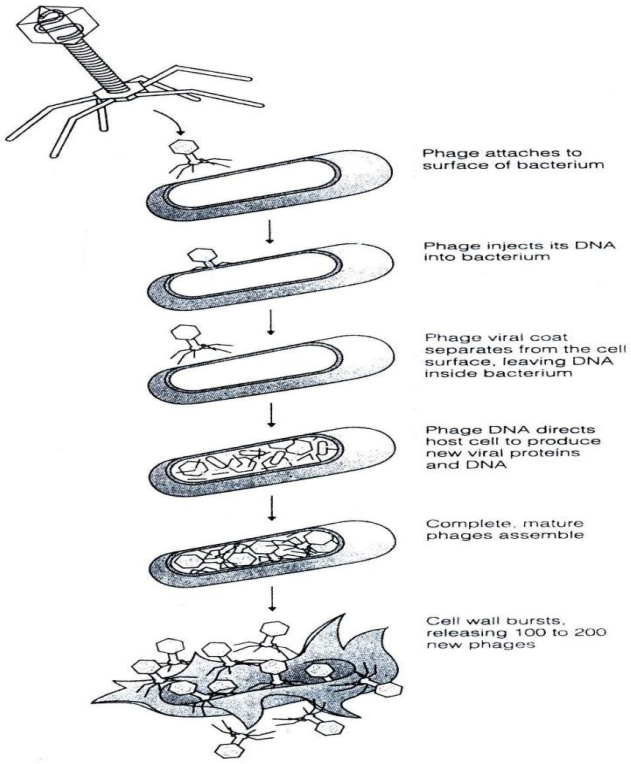

Figure 1.10: The lytic life cycle of a bacteriophase

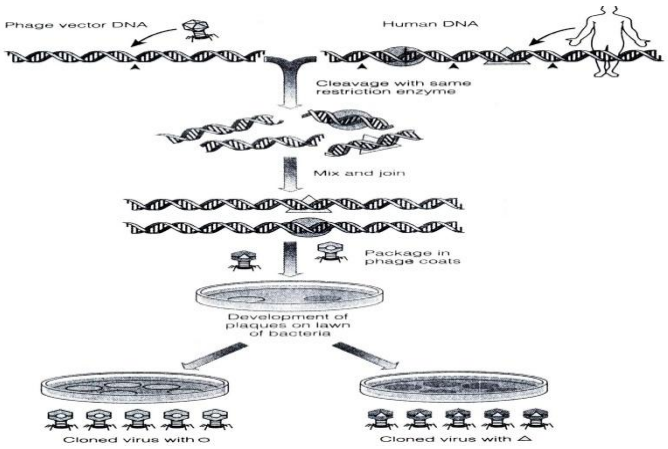

Figure 1.11: The cloning of human DNA restriction fragments (O and Δ) into a bacteriophage vector

Cosmids:

A significant disadvantage of using plasmids and bacteriophage as vectors is that only relatively small DNA fragments can be inserted. Larger DNA fragments can be cloned using engineered hybrids of phage DNA and plasmids called cosmids. These are plasmids with a small portion of lambda DNA, the cos sites. A cosmid vector not only is composed of cos sites for packaging into phage particles but also contains a plasmid replication origin for replication in

bacterial hosts and genes for plasmid selection (for example, antibiotic resistance genes to confer resistance to antibiotics such as ampicillin or kanamycin). The cosmid vector is packaged in a protein coat in vitro as with a bacteriophage vector; however, after the packaged DNA infects E. coli host cells, the DNA replicates as a plasmid rather than as bacteriophage DNA and the cells are not lysed. Bacterial colonies rather than plaques are formed on petriplates. Cosmid vectors are small (some only 2.5 kb), and because they are packaged for infection of host cells, if cos sites are separated by 37 to 52 kb, they accommodate large inserts of foreign DNA. Typically, 35 to 45 kb can be cloned into cosmid vectors.

The following compares available vectors for bacterial host cells:-

Vector	Insert Size	Characteristics
Plasmids	≤10 kb	Autonomously replicates in host cell, high or low copy number plasmids
Bacteriophage	5-20 kb	Packaged into protein coat, kills host cells, variable insert Size
Cosmids	35-45 kb	Packaged like a bacteriophage, replicates like a plasmid without killing host cells

Other Organisms:

Although many cloning experiments are carried out using E. coli as the host cells, other organisms also are used that require different types of cloning vectors. When the goal is to obtain a protein product such as insulin or growth hormone or to modify the properties of a specific organism, such as to introduce pest resistance into soybean, the cloning vector must be compatible with the organism used. Cloning vectors are available for yeast and other fungi, plants, insects, fish, and mammals.

Artificial Chromosomes Yeast artificial chromosomes (YACs) are useful for eukaryotic molecular studies. The yeast chromosome has the following necessary components:

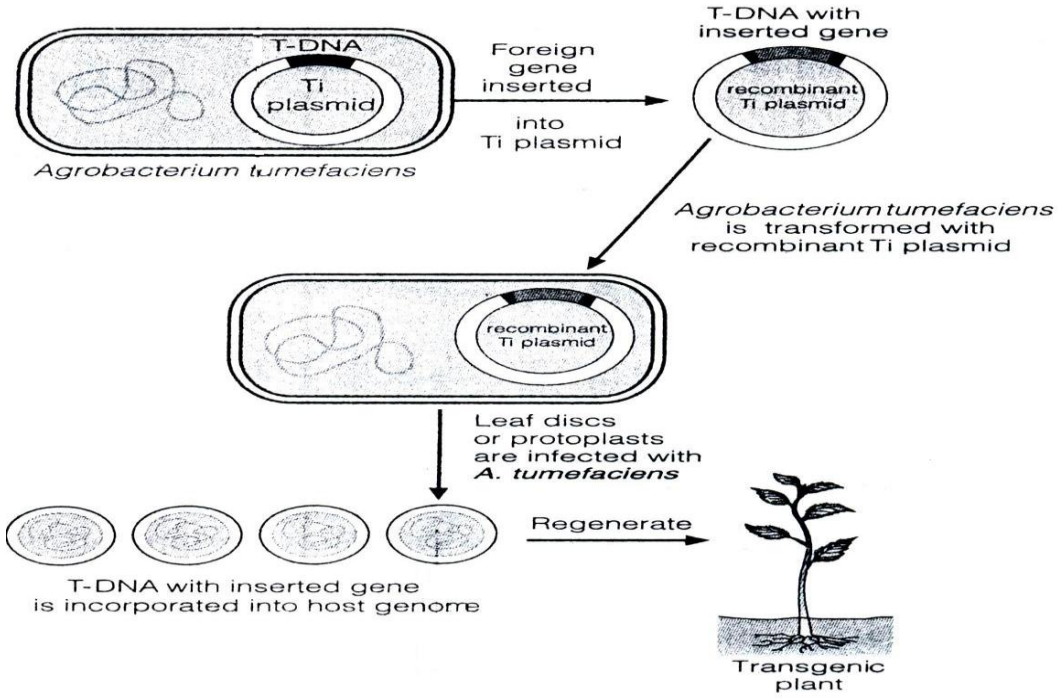

Figure 1.12: The use of *Agrobacterium tumefaciens* to transfer DNA into plants. A gene of interest is cloned into the T-DNA of the *A. tumefaciens* plasmid (along with a selectable marker gene), and *A. tumefaciens* is transformed with the recombinant Ti plasmid. Transformed *A. tumefaciens* is used to infect plant cells in culture; the T-DNA, including the foreign gene, is transferred into plant chromosomal DNA. Plants regenerated from cultured cells are tested for the presence of the foreign gene

- A centromere distributes the chromosome to the daughter cells during cell division
- A telomere at the end of the yeast chromosome ensures that the end is correctly replicated and protects against degradation
- An autonomously replicating sequence (ARS) consists of specific DNA sequences that enable the molecule to replicate. YACs also have a gene that provides a way to detect an inserted DNA fragment. These components are joined to make a YAC that can replicate in yeast host cells.

YACs are especially useful for cloning large DNA fragments. Many animal genes being studied can be 200 kb or more, requiring a cloning vector that accommodates very large fragments. As noted earlier, many vectors accommodate only rather small DNA inserts. YAC vectors accept fragments of between 200 and 1500 kb, allowing complete genes as well as gene clusters to be cloned for study. YAC libraries are available and are very useful for large

genomes, such as the human genome. They enable researchers to isolate and sequence specific regions of the genome.

Bacterial Artificial Chromosomes Bacterial artificial chromosomes (BACs) are synthetic vectors and have been the most widely used DNA cloning system for large genome sequencing projects. They have been used to clone very large fragments of eukaryotic genomes—100 to 300 kb of DNA, with the average size being 150 kb. BACs are constructed using a very low copy *E. coli* plasmid vector—the naturally occurring fertility factor plasmid—called the F factor. The F factor of *E. coli* is a circular plasmid of approximately 100 kb that encodes proteins that allow the molecule to replicate.

Results and Discussions:

MS medium fortified with 1.0 mg/l BAP or 2.0 mg/l L-Glutamic acid also induced shoot buds on stem node segments. Addition of NAA failed to produce many shoots but enlarged the stem node segments. Lower levels of coconut milk (6, 12%) induced callus formation. The results from study have shown the initiation of shoot buds and formation of multiple shoots from different explants i.e. stems Cotyledonary cuttings of *Cucurbita maxima.*

Table 1: Production of Somatic Hybrids. 1. *Pitaya roja* 2. *Solanum Melangina* 3. *Potato*, 4 & 5 *Somatic Hybrid varieties Rose and Ipomoea*

Growth Regulators	Cotyledonary Segments	
	% frequency of shoots	Mean no. of shoots
MS + 0.5 mg/l BAP + 0.5 L-Glutamic acid+Kn	45	Green Callus
MS + 1.0 mg/l BAP + 1.0 L-Glutamic acid+Kn	40	Green Callus
MS + 2.0 mg/l BAP + 2.0 L-Glutamic acid+Kn	35	Callus+shoots (1-4)
MS + 3.0 mg/l BAP + 3.0 L-Glutamic acid+Kn	30	shoots (1-3)
MS + 0.5 mg/l NAA + CM	25	Callus
MS + 1.0 mg/l NAA + CM	20	Callus
MS + 2.0 mg/l NAA + CM	15	shoots (3-6)
MS + 3.0 Mg/l NAA + CM	10	shoots (2-4)

CM = Coconut milk water

Among all explants used stem node segments were the best for multiple shoot induction. With an increase in the level of BAP 2.0 – 3.0 mg/l the percentage of explants producing shoots

also increased. The Cotyledonary segments cuttings were inoculated on M Venkateshwarlu (2019& 2020).MS basal medium fortified with various cytokinins i.e., BAP and NAA. Coconut water also had a role in triggering the formation of multiple shoots. Raising the level of BAP (3 mg/l to 4 mg/l) resulted in an increase in the percentage of shoots developed from Cotyledonary segments cuttings. There was no significant increase in the number of shoots on NAA at low and high concentration. (Table-1, Plate-1)

Plate 1: Production of Somatic Hybrids. 1. *Pitaya roja* 2. *Solanum Melangina* 3. *Potato*, 4 & 5 *Somatic Hybrid varieties Rose and Ipomoea*

Conclusion:

The enzyme DNA ligase can join DNA fragments that have complementary sticky ends or blunt ends. Ligase catalyzes the formation of covalent bonds between the sugar and the

phosphate of the adjacent nucleotides, requiring only that one nucleotide have a free 5' phosphate and the adjacent one have a 3' hydroxyl group. Ligase does not discriminate between DNAs with different origins. Thus, two DNA fragments cut from the chromosomes of two different organisms by restriction enzymes are joined by DNA ligase (Figure 1.2). The two fragments are now one DNA molecule. This cut-and-paste technique produces a recombinant DNA molecule. It is then transferred to a host cell where it is amplified (replicated) for further study. This process is called *DNA cloning* (making multiple, identical DNA copies).

Refferences:

Ahloowalia BS. Somatic embryos in monocots. Their genesis and genetic stability. Rev. Cytol. Biol. Veget. Bot. 1991; 14:223-225.

Amberger LA, Palmer RG, Shoemaker RC. Analysis of culture-induced variation in soybean. Crop Sci. 1992; 32:1103-1108

Barbara Piwowarczyk, and Anna Pindel, Determination of an optimal isolation of culture conditions of grass pea protoplasts, Biotechnologia, 96 (2), 192 – 201 (2015).

Bellincampi D, Morpugo G. Conditioning factor affecting growth in plant cells in culture; Plant Sci. 1987; 51:83-91

Beversdorf WD, Bingham ET. Degrees of differentiation obtained in tissue cultures of Glycine species. Crop Sci. 1977; 17:307-311.

Binding H. Cell cluster formation by leaf protoplasts from axenic cultures of haploid, 1974.

Butenko R, Kuchko AA. Physiological aspects of procurement, cultivation and hybridization of isolated potato protoplasts Sov. Plant Physiol. (English Transl.). 1980; 26:901-909.

Christianson ML, Warnick DA, Carlson PS. A morpho genetically competent soybean suspension culture. Science. 1983; 222:632-634.

Cocking EC. A method for the isolation of plant protoplasts and vacuoles; Nature (London). 1960; 187:927-929

Drew,R. Application of tissue culture to plant breeding, Proc. 10th Australiam Conf. on Plant Breeding, Gold Coast, Queensland, Australia, 102 – 115 (1993).

Duke JA. Handbook of legume of world Economics importance. Plenum press, New York, 1981.

Fernanda Januzzi Mendes-da-Gloria, F. de A. A. M. Filho, L. E. A. Camargo, and B. M. J.Mendes, Caipira sweet orange + Rangpur lime : a somatic hybrid with potential for use as root stock in the Brazilian citrus industry, Genet. Mol. Biol., 23(3), 661 – 665 (2000).

Gaj MD. Direct somatic embryogenesis as a rapid and efficient system for *in vitro* regeneration

of Arabidopsis thaliana. Plant Cell Tiss. Organ Cult. 2001; 64:39-46.

Ghazi TD, Cheema HV, Nabors MW. Somatic embryo genesis and plant regeneration from embryonic callus of soyabean (*Glycine max* (L). Marr.), Plant physiol. 1986; 77:863-868.

Grosser, J. W. and F. G. Gmitter Jr., Protoplast fusion for production of tetraploids and triploids: application of scion and rootstock breeding in Citrus, Plant Cell Tiss. Org. Cult., 104, 343 – 357(2011).

Handley,L. W. and T. Kumashiro, Transfer of crop traits by protoplast fusion techniques, Agri. Cell Rep., 6(6), 42 – 46 (1986).

Hansen G, Wright MS. Recent advances in the transformation of plants, Trends plant Sci. 1999; 4:226- 231.

Henry RJ, Nato A, De Buyser J. Genetic fidelity of plants regenerated from somatic embryos of cereals. In: Jain SM, Brar DS, Ahloowalia BS (EDS) Somaclonal variation and induced mutations in crop improvement. Kluwer Academic Publishers, Dordrecht, Netherlands, 1998, 65-80.

Jeff Schell., Transgenic plants as tools to study the molecular organization of plant genes, Science, 237, 1176 – 1183 (1987).

Kao, K.N. and M.R. Michayluk., Fusion of plant protoplasts – Techniques, In: Y.P.S.Bajaj (Ed.), Biotechnology in agriculture and forestry, Vol. 8 : Plant protoplasts and genetic engineering I : Springer – Verlag, Berlin (1990).

Mandaloju Venkateshwarlu (2020) Shoot induction from Trendril explants of Luffa actangula L A Vegetable crop plant. EJBPS Col.7 Issue:02, pp: 327-329

Melchers, G. and G. Labib., Somatic hybridization of plants by fusion of protoplasts. I. Selection of light resistant hybrids of „haploid" light sensitive varieties of tobacco, Mol.Gen. Genet., 135, 277 – 294 (1974).

Ramezan Rezazadeh, D. K. Harrison, and R. R. Williams, Intraspecific somatic hybridization of mango (Mangifera indica L.) through protoplast fusion, J. of App.Horti.,13(2), 101 – 107 (2011).

Roest, S. and J. W. Gilissen., Plant regeneration frim protoplasts : a literature review, Act. Bot. Neerl., 38 (1), 1 – 23 (1989).

Szarka, B. I. Gonter, M. Molnar-Lang, and M. S. Duditso, Mixing of maize and wheat genomic DNA by somatic hybridization in regenerated sterile maize plants, Theor. App. Genet., 105, 1 – 7 (2002).

Tiwari,J. K. D. Sarkar, Poonam, S. Sharma, and J. Gopal, Solanum tuberosum (+) Solanum

pinnatisectum somatic hybrids : a new source of horizontal resistance to potato late blight in India, Sol. Newsletter, 4, 4 – 10 (2011).

Tiwari,J. K. Poonam, D. Sarkar, S. K. Pandey, J. Gopal, and S. R. Kumar, Molecular and morphological characterization of somatic hybrids between Solanum tuberosum L. & Solanum etuberosum Lindl., Plant Cell Tiss. Organ Cult., 103, 175 – 187 (2010).

Tudses, N. S. Premjeet, and D. Premjet, Establishment of method of PEG mediated protoplast fusion between Jatropha curcas and Ricinus communis, Int. J. Life Sci. Biotech. & Pharma. Res., 4(1), 50 – 56 (2015).

Tudses,N. S. Premjet, and D. Premjet, Optimal conditions for high yield protoplast isolations of Jatropha curcas L. and Ricinus communis L., American – Eurasian J. Agric. Environ. Sc., 14(3), 221 – 230 (2014).

Vasil,I. K. Plant Biotechnology 2002 and beyond, Proc. 10th IAPTC & B Congress,Orlando, Florida, USA, Kluwer Acad. Publ., Dordrecht, The Netherlands(2002).

Venkateshwarlu M, Dinesh Khanna N and Ugender T. (2019) Protoplast Isolation of soybean *Glycine max* (L) Merrill from leaf explants. The Pharma Innovation Journal NAAS Rating pp: 368-373.

Xhu, C. G. Xia, D. Zhi, F. Xiang, and H. Chem, Integration of maize nuclearand mitochondrial DNA into the wheat genome through somatic hybridization, Plant Sci., 165, 1001 – 1008 (2003).

IMPORTANCE OF SPENT MUSHROOM SUBSTRATE

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Spent mushroom substrate (SMS) is the material which is left after growing mushroom.In mushroom producing regions, the spent mushroom substrate (SMS), is generated in large quantity as 1 kg of fresh mushrooms utilizes nearly 5 kg of spent substrate (Finney *et al.*, 2009). SMS are bulky products long considered a waste stream (Pardo-Giménez *et al.*, 2012). The traditional methods of disposal or burning it without any important useare neither eco-friendly nor economic (Oei *et al.*, 2007; Carrasco *et al.*, 2018) which could cause a series of environmental problems including air pollution. Therefore, it is necessary to adopt new methods for the beneficial use of SMS in improved applications.The use of spent mushroom substrate in the nutrient poor soil leads to an improved health of soil texture, improvement of water holding capacity and nutrient status. Addition of SMS in soil does not have any adverse effect on its alkalinity while, its application in soil leads to an increase in both pH as well as the organic carbon content. The phosphorus and potassium requirements of the crop plants can be fulfilled by adding 5 % of SMS by volume, while nitrogen requirement can be fulfilled by 25 % of SMS by volume.Waste water generated from many industries is polluted with heavy metals, dyes used intextile or plastic industries, or pesticides from agricultural practices. Spent substrate from Agaricusbisporus, various Pleurotus species have been evaluated for the removal of heavy metals andnumber of other contaminants successfully. Peksen and Uzun (2008) proved that spent mushroom substrates is rich in organic matter and constitutes macro and micronutrients for growth of crop plants and soil microorganisms; hence, it increases the soil microflora and enhances soil enzymatic activities. The SMShave potential benefits in many fields, like a soil-less growing medium (Medina *et al.*, 2009; Ribas *et al.*, 2009), soil and water bioremediation (García-Delgado *et al.*, 2013; Jordan *et al.*, 2008; Lau *et al.*, 2003), energy feedstocks (Finney *et al.*, 2009), animal feeds (Li *et al.*, 2001), and soil, organic amendments (Courtney and Mullen, 2008; Paula *et al.*, 2017).

In many studies it is observed that with the addition of SMS in soil not only increases the yield performance but also shows an increase the concentration of nitrogen fixing bacteria in soil. The protein content of crop, particularly oil seed crop can be increases with incorporation of

SMS and the quality of ear was improved (Sagar *et al.,* 2007). SMS mainly contain straw and this improves the drainage quality of soil and also helps in breaking down of clayey soil and thereby improves the structure of soil. SMS has high Calcium and therefore it is good for plants that require a high amount of calcium, for e.g. tomatoes. Roy *et al.* (2015) used spent mushroom substrate of oyster and button mushroom as bio-fertilizer for growth of *Capsicum annuum* and reported the positive results with improvement of health status of *Capsicum annuum in terms of protein content and yield performance.* SMS can be used for the development of various nursery plants like shrubs.In green house crops SMS can be used for production of different crops as a growing media for many seedlings. SMS can be used as mulch which increases water holding capacity and protects the seed from birds eating away. Spent mushroom substrate can be used as a liquid plant hormone and testedin different vegetables and a good yield was obtained (Hui *et al.,* 2007).Addition of SMS in soil found to be effective in checking manysoil borne diseases. *Agaricus bisporus* SMS has been assessed against various plant diseases likeapple scab, damping-off disease of Tomato, *Fusarium* wilt, rot and other soil borne diseases of many cropsand the results were highly satisfactory. Since SMS is an organic waste its utilization for disease management is environmental friendly as well as cost effective.

It reduces the intensity of pathogens and hence increases the yield of crops (Kang *et al.,* 2017; Shitole, 2018). Bioremediation is the process of removal of contaminants with the help of living organisms like fungi, bacteria. SMS contain different substances such as lignin peroxide, manganese peroxide and other compounds which are responsible for breaking the compounds like hydrocarbons and also SMS has the ability to decompose xenobiotic compounds and also the adsorption capacity of SMS where it adsorbs organic and inorganic contaminants (Mohseni and Allen, 1999; Singh *et al.,* 2011).The spent substrate of *Lentinula edodes* evaluated for degradation of metalaxyl, benalaxyl, penconazole, tebuconazole, pyrimethanil, cyprodinil, iprovalicarb, and azoxystrobin (Marín-Benito *et al.,* 2012), and chlorothalonil and imidaclopridlinuron (Gao *et al.,* 2015).

Mashrooms are rich source of protein and minerals and the substrate used for its growth may include cereal straws and various grains that are components of animal diets. Thus, investigations have pursued the recycling of SMS as a feedstock.

Reference:

Courtney, R.G., Mullen, G.J. (2008): Soil quality and barley growth as influenced by the land application of two compost types. Bioresour. Technol. 99, 2913–2918.

Carrasco, J., Zied, D.C., Pardo, J.E., Preston, G.M. and Giménez, A.P. (2018): Supplementation in mushroom crops and its impact on yield and quality. AMB Expr 8:146–156.

Finney, K.N., Ryu, C.K., Sharifi, V.N., Swithenbank, J. (2009): The reuse of spent mushroom compost and coal tailings for energy recovery: comparison of thermal treatment technologies. Bioresour. Technol. 100, 310–315.

García-Delgado, C., Jiménez-Ayuso, N., Frutos, I., Gárate, A., Eymar, E., (2013): Cadmium and lead bioavailability and their effects on polycyclic aromatic hydrocarbons biodegradation by spent mushroom substrate. Environ. Sci. Pollut. Res. 20, 8690–8699.

Gao W, Liang J, Pizzul L, Feng XM, Zhang K, Del Pilar Castillo M. (2015): Evaluation of spent mushroom substrate as substitute of peat in Chinese biobeds. *International Biodeterioration and Biodegradation,* 98:107–112.

Hui Zeng *et al*. (2007): The alternative uses of spent mushroom compost. Spore, 1-22.

Jordan, S.N., Mullen, G.J., Murphy, M.C. (2008): Compositional variability of spent mushroom compost in Ireland. Bioresour. Technol. 99, 411–418.

Kang DS, Min KJ, Kwak AM, Lee SY and Kang HW. (2017): Defense response and suppression of Phytophthora blight disease of pepper by water extract from spent mushroom substrate of *Lentinula edode*s. *The plant pathology journal*,33(3)::264.

Li, X.J., Pang, Y.Z., Zhang, R.H. (2001): Compositional changes of cottonseed hull substrate during *P. ostreatus* growth and the effects on the feeding value of the spent substrate. *Bioresour. Technol*. 80, 157–161.

Lau, K.L., Tsang, T.T., Chiu, S.W. (2003): Use of spent mushroom compost to bioremediate PAH-contaminated samples. Chemosphere 52, 1539–1546.

Mohseni M and Allen DG. (1999): Transient performance of biofilters treating mixtures of hydrophilic and hydrophobic volatile organic compounds. *Journal of the Air & Waste Management Association*,49(12)::1434-1441.

Medina, E., Paredes, C., Pérez-Murcia, M.D., Bustamante, M.A., Moral, R. (2009): Spent mushroom substrates as component of growing media for germination and growth of horticultural plants. Bioresour. Technol. 100, 4227–4232.

Marín-Benito JM, Rodriguez-Cruz MS, Andrades MS, Sanchez-Martin MJ. (2012): Assessment of spent mushroom substrate as sorbent of fungicides: influence of sorbent and sorbate properties.*Journal of Environmental Quality*,41:814–822.

Oei, P., Hui, Z., Jianhua, L., Jianqing, D., Meiyuan, C., Yi, C. (2007): The alternative uses of spent mushroom compost. In: Proceedings of the 6th international conference on

mushroom biology and mushroom products, Bonn, Germany, 29 September–3 October, pp 231–245.

PeksenA and S. Uzun. (2008): Effects of spent mushroom compost on growth and nutrient contents of pepper seedlings," *Horticultural Society*, 108: 232–234.

Pardo-Giménez A, Picornell Buendia MR, de Juan Valero JA, Pardo-Gonzalez JE, Zied CD (2012): Cultivation of *Pleurotus ostreatus* using supplemented spent oyster mushroom substrate. Acta Hortic 933:267–272. https://doi. org/10.17660/ActaHortic.933.33.

Paula, F.S., Tatti, E., Abram, F., Wilson, J., O'Flaherty, V. (2017): Stabilisation of spent mushroom substrate for application as a plant growth-promoting organic amendment. J. Environ. Manag. 196, 476–486.

Ribas, L.C.C., de Mendonça, M.M., Camelini, C.M., Soares, C.H.L. (2009): Use of spent mushroom substrates from Agaricus subrufescens (syn. A. blazei, A. brasiliensis) and Lentinula edodes productions in the enrichment of a soil-based potting media for lettuce (lactuca sativa) cultivation: growth promotion and soil bioremediation. Bioresour. Technol. 100, 4750–4757.

S. Roy, B. Shibu, U. Chakraborty, and B. Chakraborty. (2015): "Evaluation of spent mushroom substrate as bio-fertilizer for growth improvement of *Capsicum annuum* L," *Journal of Applied Biology and Biotechnology*, 3(3): 22–27.

Sagar MP, Ahlawat OP, Raj D, Vijay B, Indurani C. (2007): Indigenous technical knowledge about the use of spent mushroom substrate.

Shitole, Amol Vijay, Gade RM, Bandgar MS, Archana Zalte. (2013): Utilization of spent mushroom substrate for management of tomato damping off.*J Plant Dis. Sci.*, 8:196-9 and Biofertilizer." The Bioscan 2014;9(1):271-275.

Singh Avneesh D, Noorlidah Abdullah, Vikineswary S. (2003):Optimization of extraction of bulk enzymes from spent mushroom compost.*Journal of Chemical Technology & Biotechnology: International Research in Process, Environmental & Clean Technology*, 78(7):743-752.

OMICS TECHNOLOGIES FOR STANDARDIZATION AND AUTHENTICATION OF ADVANCES IN PLANT SCIENCE

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Abstract:

The results of the integrated omics approach can help us better understand how plants' physiology changes as environmental conditions change. These omics tools generate a lot of biological data, which requires a lot of analysis to figure out gene functions, genome organization and structure, complex plant molecular pathways, and regulatory networks that control plant growth and biotic/abiotic interactions under various physiological, pathological, or environmental conditions. The functions, interactions, and activities of the various types of molecules that make up an organism's/cells are investigated using omics technology.

Introduction:

Informally, the English neologism omics refers to a discipline of biology that ends in -omics, such as genomics, proteomics, or metabolomics. The related suffix -ome is used to refer to the research objects of such domains as the genome, proteome, or metabolome. In molecular biology, the suffix -ome refers to a totality of some form; thus, the term omics has evolved to refer to the study of big, comprehensive biological data sets.The "omic " technologies are high-throughput technologies that significantly enhance the number of proteins/genes that can be identified simultaneously in order to link complex mixtures to complex effects via gene/protein expression patterns. The primary goal of omic technologies is to identify all gene products (transcripts, proteins, and metabolites) present in a biological sample without being targeted. Biomarker discovery, identification of signalling molecules related with cell development, cell death, cellular metabolism, and early cancer detection have all benefited from the strong 'omics' technology. Omics technologies used to explore the roles, relationships, and actions of the various types of molecules that make up the cells of an organism/plant.

Genomics, “the study of genes and their function”

Proteomics, the study of proteins

The study of chemicals involved in cellular metabolism is known as metabolomics.

Transcriptomics, the study of the mRNA

Glycomics, the study of cellular carbohydrates

Lipomics, the study of cellular lipids

Omics technologies are used to investigate the functions, interactions, and activities of the numerous sorts of molecules that make up an organism's/cells.

Plant's Genomics

1] Genomics:

Genomics is a branch of science that tries to comprehend an organism's full genetic information about sequence, assemble, and analyse the structure and function of genomes, genomics use a combination of recombinant DNA, DNA sequencing technologies, and bioinformatics. Information on the human genome: It refers to the information stored in DNA (deoxyribonucleic acid) and RNA (ribonucleic acid). It will be a huge accomplishment to characterise the identity, function, regulation, and interaction of all of an organism's cellular proteins, known as the proteome. To obtain a better knowledge of the processes of toxicity, researchers are looking at alterations in the proteome of cells and tissues exposed to hazardous chemicals compared to normal cells. Protein and proteome changes in response to toxic substance exposures (fingerprints or response profiles) will be developed into databases that can be used to classify exposure responses at various levels of organisation of the organism, providing a predictive in silico toxicology tool as proteomics tools become more powerful and widely used.

Genome analysis:

Genome projects have three components after an organism has been chosen: DNA sequencing, assembly of that sequence to generate a representation of the original chromosome, and annotation and analysis of that representation.

a. Sequencing:

Previously, sequencing was done in sequencing centres, centralised facilities (ranging from large independent institutions like the Joint Genome Institute, which sequences dozens of terabases per year, to local molecular biology core facilities) that housed research laboratories with the necessary expensive instrumentation and technical support. However, as sequencing technology advances, a new generation of efficient fast turnaround benchtop sequencers has

become affordable to the average university laboratory. Shotgun and high-throughput (or next-generation) sequencing are the two primary categories of genome sequencing techniques.

b. Assembly:

The process of matching and combining pieces of a much longer DNA sequence in order to recover the original sequence is known as sequence assembly. This is necessary since present DNA sequencing technology cannot read entire genomes in a continuous sequence, but rather reads short chunks of DNA ranging from 20 to 1000 bases, depending on the technology. Third-generation sequencing technologies like PacBio and Oxford Nanopore frequently produce sequencing reads longer than 10 kb, although they have a high error rate of about 15%. Shotgun sequencing of genomic DNA or gene transcripts produces tiny pieces known as reads (ESTs).

c. Annotation:

Without further study, the DNA sequence assembly is of little use. The process of attaching biological information to sequences is known as genome annotation, and it consists of three main steps: identifying portions of the genome that do not code for proteins, identifying elements on the genome, a process known as gene prediction, and identifying elements on the genome.

Biological information is attached to these pieces.

In contrast to manual annotation (a.k.a. curation), which requires human skill and potentially experimental verification, automatic annotation programmes attempt to accomplish these stages in silico. These methodologies should ideally coexist and compliment one another in the same annotation workflow.

Traditionally, the most basic level of annotation involves discovering homologues using BLAST and then annotating genomes based on those homologues. More information has recently been added to the annotation platform. Manual annotators can use the additional information to deconvolute differences between genes that have the same annotation. Through their Subsystems method, some databases provide genome annotations using genomic context information, similarity scores, experimental data, and integrations of other resources. In their automated genome annotation workflow, other databases (such as Ensembl) rely on both curated data sources and a variety of software tools. The identification of genomic elements, especially ORFs, and their localization, or gene structure, is referred to as structural annotation. The process of connecting biological information to genomic sites is known as functional annotation.

2] Transcriptomics:

The transcriptome, or all of the RNA molecules in a cell, is the subject of transcriptomics.It is the study of an organism's transcriptome. The transcriptome is the collection of all RNA molecules produced in one or more cells, including mRNA, rRNA, tRNA, and non-coding RNA. Transcriptomics, also known as expression profiling, is a technique for determining the level of RNA expression in a population of cells.RNA comes in a variety of forms. The most common form, known as messenger RNA (mRNA), is essential for protein synthesis. The following steps are involved in this process: mRNA is transcribed from genes; the mRNA transcripts are then given to ribosomes, which are molecular machines found in the cell's cytoplasm; and finally, the ribosomes are carried to the cell's nucleus.

- **Transcriptomics Analysis :**

To aid in the discovery of genes that are differentially expressed in different cell types, a number of organism-specific transcriptome databases have been built and annotated.

Although the earlier approach of DNA microarrays is still utilized, RNA-seq is emerging (2013) as the method of choice for quantifying transcriptomes of organisms.

By transforming lengthy RNAs into a library of cDNA fragments, RNA-seq assesses the transcription of a specific gene. After that, the cDNA fragments are sequenced using high-throughput sequencing technology and aligned to a reference genome or transcriptome, which is then used to build a gene expression profile.

3] Metabolomics:

For metabolite profiling, a variety of approaches are employed. Each method has its own set of benefits and cons. To acquire a broad perspective on a tissue's metabolome, a combination of several analytical methodologies must be applied. The study of the metabolite composition of a cell type, tissue, or biological fluid is known as metabolomics. For more than a decade, biological researchers have been analyzing the entire set of metabolites (the metabolome) (Patti *et al.,* 2012). However, significant recent advancements in the technologies used to extract and interpret this type of molecular data have changed its applicability in the study of organisms and biological processes (Zhang *et al.,* 2012). To present, metabolomics is regarded as one of the most important "omics" technologies for achieving difficult research goals such as treatment personalisation in clinical practise.

- **Metabolomics Analysis:**

The most commonly utilised techniques are NMR, gas chromatography–mass spectrometry (GC–MS), liquid chromatography–mass spectrometry (LC–MS), and capillary electrophoresis–mass spectrometry (CE–MS). NMR is well-suited to metabolomics studies

because it can uniquely identify and quantify a large range of organic molecules in the micro-molar range. For metabolite fingerprinting, profiling, and metabolic flux, NMR has been widely used.

4] Proteomics:

Proteomics is the study of the proteome, which looks at how different proteins interact with one another and what roles they perform in the body.

Although the expression of mRNA, the intermediary between genes and proteins, can be used to infer protein expression, mRNA expression levels do not necessarily correspond well with protein expression levels.

Furthermore, mRNA research ignores protein posttranslational modifications, cleavage, complex formation, and localization, as well as the numerous different mRNA transcripts that can be produced, all of which are important for protein function.

With the development of 2D protein electrophoresis in 1975, the first investigations that meet the label of "proteomic" studies were done.

However, it was not until the invention of mass spectrometry (MS) technology over 20 years later that true high-throughput identification of many proteins per sample became conceivable.

Since then, MS has improved in sensitivity and accuracy to the point that proteins can now be successfully detected at attomolar concentrations (1 target protein molecule per 1018 molecules), and numerous other proteomic techniques have been created and enhanced.

- **Proteomics Analysis :**

Proteome analysis is the separation, identification, and quantification of a genome's, cell's, or tissue's full protein complement (Wasinger *et al.*, 1995; Wilkins *et al.*, 1996). Proteomics has evolved into a strong tool for analysing complex protein mixtures employing two primary techniques: 2-DGE (Patterson and Aebersold, 1995) and MS (Aebersold and Mann, 2003). HPLC is a different type of separation technology used in proteomic investigations, particularly for separating and identifying low-molecular-weight proteins and peptides (Garbis *et al.*, 2005)

Proteomics procedures can alternatively be "bottom-up" or "top-down," in which proteins are identified from their protease (e.g., trypsin) digests or as a whole using a mass spectrometer. The protein is either sequenced de novo by manual mass analyses of the spectra or processed automatically by sequence search engines such as SEQUEST, Mascot, Phenyx, X!Tandem, and OMSSA using the data provided by the MS. These algorithms are based on the correlation of

experimental and theoretical MS/MS data, the latter of which is derived from in silico digestion of protein databases such as UniProt/Swiss-Prot (Deutsch, Lam, and Aebersold, 2008). Since gel electrophoresis and LC methods for separation and detection of whey proteins/peptides have already been covered, this section will focus on MS as the most commonly used instrument in proteomics. The mass to charge ratio (m/z) of ions in the gas phase allows MS to determine the molecular mass of proteins or peptides. A mass spectrometer has three basic components: an ion source that produces ions and transfers them to the gas phase, a mass analyzer that measures the m/z of these ions, and a detector that counts the quantity of ions at each m/z value. Electrospray ionisation (ESI) and MALDI are the two most frequent ion sources in proteomics.

5] Glycomics:

The term glycome refers to the total glycan(carbohydrate) component present in live cells of organisms, both free and mixed. The systematic study of the glycan structures found in any living cell is thus termed as glycomics. Glycomics is concerned with the study of carbohydrates in combination with or related with other macromolecules, such as lectins, glycoproteins, glycolipids, carbohydrate-specific enzymes, carbohydrate-specific cell receptors, and sugar molecules found in nucleic acids. Glycomics is regarded as one of the most difficult aspects of living cell research.

- **Glycomics Analysis:**

Mass spectrometry is a technique for determining the composition of a Mass spectrometric glycomic analysis is fundamentally more difficult than glycoproteomic analysis. Identification of glycan structures is difficult due to structural differences in glycan connections and the similar mass of epimeric monosaccharides. The steps in mass spectrometric analysis of glycans are usually as follows: 1) PNGase F treatment (for N-linked glycans) or -elimination glycan release (for O-linked glycans) 2) isolation of glycans by solid-phase separation techniques 3) glycan derivatization and ionisation, followed by 4) annotation of the resultant spectra to identify potential glycan structures. Significant progress has been achieved in the isolation, derivatization, and ionisation procedures utilised for glycomic analysis in recent years. These methods improved reproducibility and significantly shortened the time required for glycomics by mass spectrometry. However, spectral data interpretation remains a fundamental obstacle for high-throughput research. This is owing to glycan mass degeneracy as well as the complex fragmentation patterns that result from the more advanced MS techniques needed to extract precise structural information. Although applications such as Cartoonist, GlycoPep DB, and SysBio Ware exist to automate some parts of data interpretation, software-based analysis of glycan spectrum data is still in its infancy. Mass spectrometry has become a commonly utilised

approach for detecting glycan epitopes in a variety of samples, including bacterial and mammalian cells, sera, and tissue samples, thanks to recent improvements in the field.

6] Lipomics:

Lipidomics is the study of cellular lipid pathways and networks at a large scale in biological systems. The term "lipidome" refers to a cell's, tissue's, organism's, or ecosystem's entire lipid profile. It is a subset of the "metabolome," which also comprises the three other major groups of biological molecules: proteins/amino acids, carbohydrates, and nucleic acids. Rapid advances in technologies such as mass spectrometry (MS), nuclear magnetic resonance (NMR) spectroscopy, fluorescence spectroscopy, dual polarisation interferometry, and computational methods, combined with recognition of the role of lipids in many metabolic diseases such as obesity, atherosclerosis, stroke, hypertension, and diabetes, have fueled the field of lipidomics. This fast growing discipline complements the enormous progress made in genomes and proteomics, which are all part of the systems biology family. The identification and quantification of the thousands of cellular lipid molecular species, as well as their interactions with other lipids, proteins, and other metabolites, are all part of lipidomics research. Lipidomics researchers study the structures, functions, interactions, and dynamics of cellular lipids, as well as the changes that occur when the system is perturbed.

- **Lipomics Analysis :**

Targeted lipidomics is a lipidomics approach that focuses on a small set of predetermined lipid-specific signals to determine their relative abundances precisely and reliably. Untargeted lipid analysis, on the other hand, seeks to detect all lipid species present in a sample at the same time, yielding a massive number of molecules to investigate. It must be used in conjunction with chemometric approaches to extract useful information from relevant signals, which is then discovered through database searches. Due to the inability to apply internal standards for quantification, it frequently necessitates very specialised and expensive software to visualize the data, and it is usually semiquantitative. The advantage of the untargeted approach is that it has the potential to uncover novel molecules of interest, with only the sample preparation procedure and analytical techniques used limiting it. As a result, global lipidomics methods are frequently used in hypothesis-generating investigations. However, for lipid classes present at low quantities in biological materials or characterized by instability or other physicochemical properties that limit the analytical approach, specific focused methods are necessary (e.g., bile acids, steroid lipids, specific signalling lipids, and lipids involved in immune system regulation). The complexity of data processing and the need to identify the compounds detected are the key

disadvantages of untargeted lipidomics. Targeted lipidomics, on the other hand, has a lower level of data manipulation complexity, but it requires prior knowledge of the lipids contained in the sample.

Conclusion:

The results of the integrated omics approach can help us better understand how plants' physiology changes in response to changing environmental conditions. These omics tools generate a large amount of biological data that requires extensive analysis to determine gene functions, genome organization and structure, complex plant molecular pathways, and regulatory networks that govern plant growth and biotic/abiotic interactions under various physiological, pathological, or environmental conditions.

References:

Assis, A. F., Oliveira, E. H., Donate, P. B., Giuliatti, S., Nguyen, C., & Passos, G. A. (2014). What is the transcriptome and how it is evaluated?. In Transcriptomics in Health and Disease (pp. 3-48). Springer, Cham.

Baker M (14 September 2012). "Benchtop sequencers ship off" (Blog). Nature News Blog.

Beynon RJ. The dynamics of the proteome: strategies for measuring protein turnover on a proteome-wide scale. Brief Funct Genomic Proteomic. 2005;3(4):382-390. doi:10.1093/bfgp/3.4.382.

Dell A, Morris HR. Glycoprotein structure determination by mass spectrometry. Science. 2001;291:2351–2356. doi:10.1586/epr.09.81.

Eisen JA (July 2012). "Badomics words and the power and peril of the ome-meme". GigaScience. 1 (1):6. doi:10.1186/2047-217X-1- 6.

Garrels JI. Proteome. In: Brenner S, Miller JH, eds. Encyclopaedia of Genetics. London: Academic Press; 2001:1575-1578.

Griffiths W, Koal T, Wang Y, Kohl M, Enot D, Deigner H. Targeted metabolomics for biomarker discovery. Angew Chem Int Ed Engl. 2010;49:5426-45 pubmed publisher

Haslam SM, North SJ, Dell A. Mass spectrometric analysis of N- and O-glycosylation of tissues and cells. Curr Opin Struct Biol. 2006;16:584–591.

Hotz RL (13 August 2012). "Here"s an Omical Tale: Scientists Discover Spreading Suffix". Wall Street Journal. ISSN 0099-9660. Retrieved 2013-01-04.

Mahal LK. Glycomics: towards bioinformatic approaches to understanding glycosylation. Anticancer Agents Med Chem. 2008;8:37–51.

Morelle W, Canis K, Chirat F, Faid V, Michalski JC. The use of mass spectrometry for the proteomic analysis of glycosylation. Proteomics. 2006;6:3993–4015.

Morelle W, Michalski JC. Analysis of protein glycosylation by mass spectrometry. Nat Protoc. 2007;2:1585–1602.

Morelle W, Michalski JC. Glycomics and mass spectrometry. Curr Pharm Des. 2005;11:2615–2645.

North SJ, Hitchen PG, Haslam SM, Dell A. Mass spectrometry in the analysis of N-linked and O-linked glycans. Curr Opin Struct Biol. 2009

Pevsner J (2009). Bioinformatics and functional genomics (2nd ed.). Hoboken, NJ: Wiley-Blackwell.

Quail MA, Smith M, Coupland P, Otto TD, Harris SR, Connor TR, Bertoni A, Swerdlow HP, Gu Y (July 2012). "A tale of three next generation sequencing platforms: comparison of Ion Torrent, Pacific Biosciences and Illumina MiSeq sequencers". BMC Genomics. 13:341. doi:10.1186/1471-2164-13-341.

Scudellari M (1 October 2011). "Data Deluge". The Scientist. Retrieved 2013-01-04.

Timp W, Timp G. Beyond mass spectrometry, the next step in proteomics. Sci Adv. 2020;6(2):eaax8978. doi:10.1126/sciadv.aax8978.

Vinayavekhin N, Saghatelian A. Untargeted metabolomics. Curr Protoc Mol Biol. 2010;Chapter 30:Unit 30.1.1-24

Wang, Z., Gerstein, M., & Snyder, M. (2009). RNA-Seq: a revolutionary tool for transcriptomics. Nature reviews genetics, 10(1), 57.

Wang, Zhong; Gerstein, Mark; Snyder, Michael (January 2009). "RNA-Seq: a revolutionary tool for transcriptomics". Nature Reviews Genetics. 10 (1): 57: 63. doi:10.1038/nrg2484.

Wilkins M. Proteomics data mining. Expert Rev Proteomics. 2009;6(6):599-603.

Xie, J., Zhang, A., & Wang, X. (2017). Metabolomic applications in hepatocellular carcinoma: toward the exploration of therapeutics and diagnosis through small molecules. RSC Advances, 7(28), 17217-17226.

Zaia J. Mass spectrometry and the emerging field of glycomics. Chem Biol. 2008;15:881–892.

Zaia J. Mass spectrometry of oligosaccharides. Mass Spectrom Rev. 2004;23:161–227.

PLANT NODAL ANATOMY – AN APPROACH

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In plants, an anatomical point of view, the place from where leaf traces arise is referred to as node.Nodes and internodes are distinct in the stem of Angiosperms.

The distinction of node in case of monocots is very difficult due to presence of scattered vascular bundles while it is well distinct among dicotyledonous stems where vascular bundles are arranged in a ring. The vascular cylinder is more complex in nature because of the presence and formation of leaf and branch traces. The vascular cylinders are generally continuous at the internode and their continuity is interrupted at the nodal region due to emergence of bundles that terminate either at the leaf bases, axillary buds or stipules, etc. At the node three types of bundles are recognized–

(i) Leaf trace bundle: The single vascular bundle that connects the leaf base with the main vascular cylinders of stem is designated as leaf trace bundle. In a leaf there may be several leaf trace bundles that collectively are termed as leaf traces.

(ii) Cauline bundle: The vascular bundles that entirely form the vascular system of stems is known as cauline bundles. Sometimes these bundles anastomose with each other and extend from stem to leaf as leaf traces.

(iii) Common bundle: The vascular bundles, which run unbranched through a few successive nodes and internode and ultimately terminate as leaf traces are called common bundles. The detailed histology of the vascular bundles may be different at the node, the cortical and the pith cells may be shorter, and there may be less sclerenchyma and more collenchyma than in the internodes (Prunet, 1891). In some herbaceous plants the main interconnections among the vertically oriented bundles occur by means of horizontally oriented strands in the nodal region.

Leaf traces and leaf gaps:

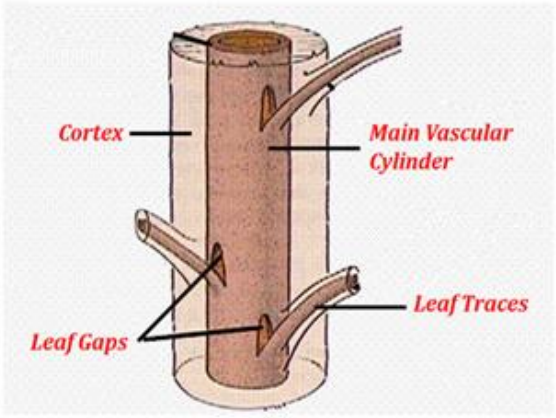
Leaf traces:

In the nodal region, a portion of vascular cyclinder is diverted towards the leaf or a bud which is attached at this node. The diverted vascular tissues form the leaf trace.A leaf trace

represents the lower part of the vascular supply of the leaf.A leaf trace may also be defined as a vascular bundle that connects the vascular bundle of the leaf with that of the stem. However one or more leaf traces may be associated with each leaf and a leaf trace may traverse one or more nodes before it diverges towards the leaf.

Leaf gaps:

In higher plants such as ferns and seed plants, with the divergence of the leaf traces immediately above the diverging traces, a region of parenchyma occurs in the vascular cyclinder of shoot. This parenchymatous region is called leaf gap. So in a leaf gap, the parenchyma is differentiated instead of vascular tissue, in the vascular region of the stem, for a limited distance. It is located adaxially from the diverging leaf traces. Actually, the leaf gaps are not breaks in vascular system because lateral connections between the vascular tissues occur above and below the gap.

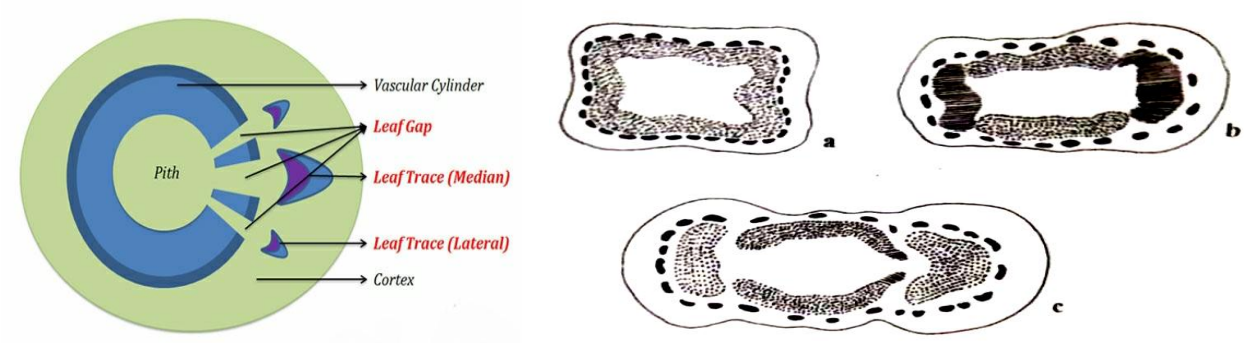


Leaf Gaps and Leaf Traces

In some ferns like *Petridium and Petris***,** the leaves are so crowded that the gaps formed at the successive nodes overlap one another and that the actual vascular cyclinder appears highly dissected. The transvers sections of such rhizomes show a circle of vascular bundles alternating with the parenchymatous leaf gaps. In certain gymnosperms, and angiosperms, the vascular systems consists of several anastomosing strands so much so that leaf traces become confluent with the interfascicular areas and then the leaf gaps become uncertain and cannot be recognize **Nodes.** There are three common types of nodes found in Dicotyledons:

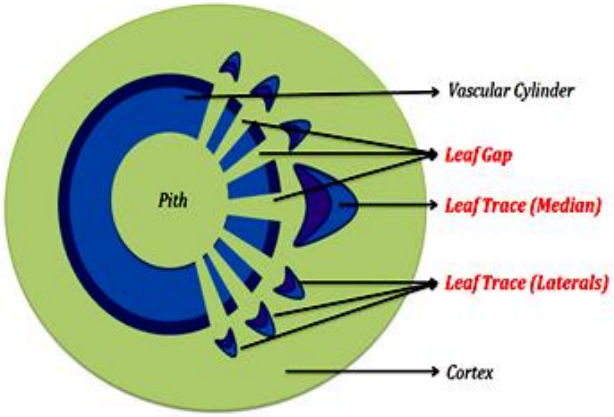
1. **Unilacunar**, a node with a single gap and a single trace to a leaf,

   Ex., Nerium, Lantana. Vitex negundo.



Ex T.S of Node of Vitex negund

2. **Trilacunar,** a node with three gaps and three traces to a leaf (one median and Two laterals).
Ex.: ***Azadirechta indica***



Multilacunar Node with Many Leaf Traces

3. **Multilacunar**, a node with several gaps and traces associated with a single leaf.
Ex.: ***Coriandum sativum, Polygonum plebeium***

In most monocotyledons, leaves with sheathing leaf bases are found which receive a large number of leaf traces separately instead around the stem.

In gymnosperms, unilocunar node is common. In ferns, variable number of leaf traces enters in a leaf but they are always associated with a single gap.

Among dicotyledons, the leaf trace relationships at the nodes are thought to be of phylogenetic importance and that is why studies on nodal anatomy becomes valuable for systematic.

Unilacunar nodes are found characteristically among members of several families such as Annonaceae, Apocyanaceae, Ericaceae, Lauraceae, Solanaceae and Verbenaceae. In some cases, one to many traces arising from a single unilocunar node have been found and accordingly these

may be called unilacunar one trace, unilacunar two traces, unilocunar three traces and unilacunar multitraces, respectively.

Trilacunar nodes are characteristically found among members of Meliaceae, polygonaceae, Ranunculaceae and Winteraceae.

Among the members of Amentiferace, Trilacunar nodes are found.

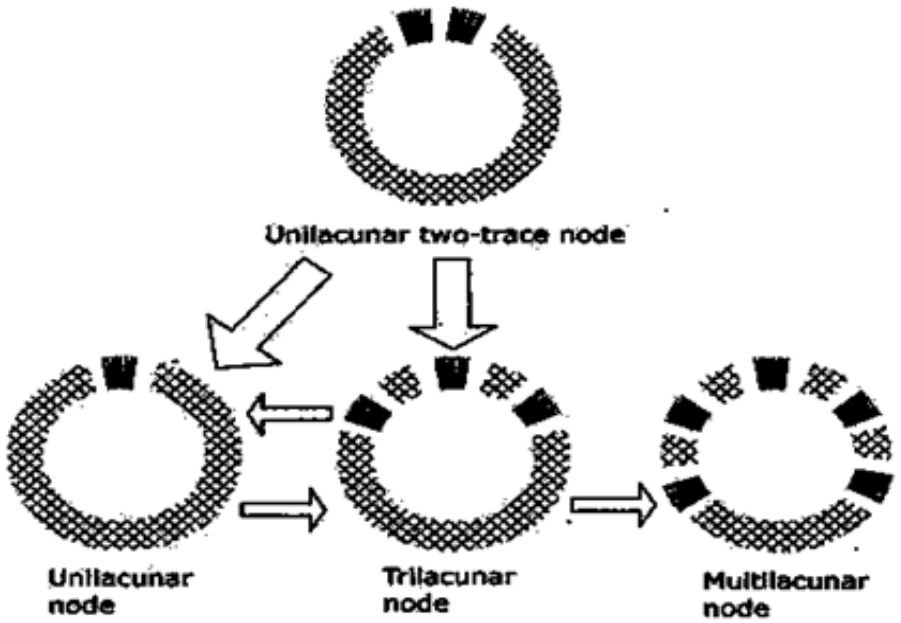
Multilacunar nodes are found among members of Araliaceae, Chenopodiaceae and Degeneriaceae.

Later in the light of above facts it is now interpreted that the evolution of nodal structure proceeded in the following two sequences:

(i) Two trace unilacunar gave rise to trilacunar, which terminated in multilacunar condition;

(ii) Two-trace unilacunar, by the loss of one trace, gave rise to one trace unilacunar that formed trilacunar node by the addition of two new gaps associated with two traces.

The multilacunar condition is derived from the trilacunar type by the addition of more new traces and gaps (Fig.) . This evolutionary sequence may be observed in a single family namely Chenopodiaceae. The trilacunar type may also give rise to one trace unilacunar condition.



Diagrams illustrating the probable lines of development of nodal vascularization

Branch traces and Branch gaps:

The vascular supply to axillary bud or lateral branches also receives vascular traces derived from the vascular system of main axis. These traces are called branch traces or ramular

traces. The vascular supply of axillary branch usually consists of two traces but in some plants either only one or more than traces are found. In monocotyledons, the connection of the axillary shoot with the main stem consists of many strands.

In the survey of the nodal organization in angiosperms, Sinnott (1914) has opined that the Verbenaceae possess a unilacunar single traced node.

Marsden and Bailey (1955) reported unilacunar two trace condition of node in Clerodendrum. Esau (1965) has also shown unilacunar two trace condition of node in this genus. According to Shah et al. (1972) the unilacunar node is with one, two or many traces condition in this family and in the species of Clerodendrum. Cronquist (1981) also indicated unilacunar nodes with 2-many traces for Verbenaceae.

References:

Cronquist, A. (1981): An Integrated system of Classification of Flowering plants,Columbia University Press, New York.

Cutler, D.F. (1978): Applied plant Anatomy, Longman, London, New York.

David F. Cutler, Ted Botha, Dennis Wm. Stevenson (20080: Plant Anatomy: An Applied Approach, 2008, Wiley–Blackwell Publishing,

Esau, K. (1965): Plant Anatomy, John Wiley and Sons, New York.

Marsden, M.P.F. and I.W. Baily (1955): A fourth type of nodal anatomy indicotyledons, illustrated by Clerodendron trichotomumThunb. J. Arnold Arb. 36:1-51.

Shah et al. (1972): Vascular supply of axillary buds in some dicotyledons. Adv. Pl. Morph. (prof. Puri Commemoration Vol.) , ed. Y. S. Murty, B.M. Johri, H.V. Mohan Ram and T. M. Varghese, Sarita Prakashan, Meerut, PP.65-75.

Sinnott, E. W. (1914): Investigations on the phylogeny of the angiosperms-I.The anatomy of the as an aid in the classification of angiosperms. Amer. J. Bot., 1: 303-332.

AGRO MEDICO STUDY OF MELGHAT REGION

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Abstract:

Melghat is known as paradise of Vidharbh .Melghat means the 'meeting of Ghats 'which is just what the area is, a large tract of unending hills .The forest of Melghat is mostly of the Dry Mixed Deciduous type and one of the important forests of Vidharbh region .The Melghat forest has great diversity of medicinal plants. Present study includes the study of agro medico study of this region.

Keywords: Medicinal plants, Melghat Forest.

Introduction:

The forest of Melghat is mostly of the Dry Mixed deciduous type and one of the important forests of Vidarbha region of Maharashtra in India. The vegetation varies considerably with the change in altitude, soil, temperature, humidity and rainfall.

The average rain fall varies from 1300 mm to 1450 mm, the temperature range varies from 13 to 41^0C and humidity varies from 48% to 100 %. The soil is also different types. The general floristic study of Melghat Forest includes the plants like 94 tree species, 708 shrubs, 368 small herbs, 66 climbers, 2 species of Bamboo, and 127 species of grasses.

The study of medicinal plants is carried out by Botanical experts, students of Agriculture University and Ayurveda timely and explored by them. Now it is necessary to study the Agro Medico View by the Expert. The farmer can do the farming of these medicinal plants.

Observation:

Sr. No.	Name of Plants	Common Name	Family	Medicinal Uses
1	*Adhatoda vasica*	Adulsa	Acanthaceae	Asthama, skin diseases, fever
2	*Cassia fistula*	Amaltas	Caesalpinoidae	Digestive problem, piles
3	*Withania somnifera*	Ashavgandha	Solanaceae	Joint pain, energetic, usable in male sexual problems
4	*Azadirachata indica*	Kadunimb	Meliaceae	Skin diseases**,** fever, snake bite, Hair problems, cosmatics, and moisquito replient
5	*Terminalia belerica*	Behada	Combretaceae	Cough, cold, asthama, digestive problems, one of the important ingredients of Triphala churn
6	*Terminalia chebula*	Hirda	Combretaceae	Increases resistance power, skin diseases, piles, digestive problems
7	*Aegle marmelos*	Bel	Rutaceae	Usable in dysentery Ulcer
8	*Phyllanthus amarus*	Bhuiawla	Euphorbiaceae	Fever
9	*Semicarpus anacardium*	Biba	Anacardiaceae	Arthritis , oil uses in joint pain
10	*Cymbopogon martini*	Tikhadi	Poaceae	Cosmatics, perfums, oil uses in paralysis and for massage
11	*Phyllanthus emblica*	Awla	Euphorbiaceae	Increases resistance power, source of Vitamin C, one of the important ingradient of Triphala
12	*Madhuca indica*	Moha	Sapindaceae	Arthritis, piles, skin diseases, usable in diabetis
13	*Mentha viridis*	Pudina	Lamiaceae	Oil used in pharmaceutical industries
14	*Catharanthus roseus*	Sadaphuli	Apocynaceae	Roots usable in diabetis and cancer treatment
15	*Asparagus racmosus*	Shatavari	Liliaceae	Energetic, Usable in ladies problem
16	*Ocimum sanctum*	Tulas	Lamiaceae	Holi plant for Hindus, Uses in Cough, cold, fever, skin diseases, headache
17	*Ruta graveolens*	Sitap	Rutaceae	Uses on worms for the childrens
18	*Dendrocalamus strictus*	Bambu	Poaceae	Use as a vegetable, Adivasi people make the Bamboo prickle Its usable in Agricultural furniture, home furniture
19	*Dioscoria tuberosa*	Babra	Dioscoreaceae	Energetuc, used as a vegetable by tribes,

Discussion:

Melghat region has Agricultural ecosystem in Melghat rural area composed of annual cultivated crops with reference to sustabitlity of soil. The average rain fall of Melghat area is 1200 -1300mm. Soil is red, murmi. The crops are in annual pattern. The tribes of Melghat Korku, Bhill, gavlon used the forest minor produce with relation to ethnobotany and ethnovetarnary aspects. In agricultural ecosystem, some of the herbaceous and busy weeds are considered as the medicinal plants and they are used in the remedy of various diseases of domestic cattle and human being.

Some of the plants are antipyurtic, laxative, used in gastric problems. From the ancient era it is observed that the culture and tradition of tribes shows that agricultural ecosystem and forest ecosystem shows close resemblance with each other and they shows the socio eco potential.Forest produce used by the tribs like bamboo, Sagvan for agriculture instruments, for their huts etc.tribes totally dependen on the forest for their life.

Referneces:

Bhogaonkar R.Y. (2002): Some unique Ethnomedicinal plants of Korkus of Melghat Tiger Reserve (MH)

Dhore, M.A. and P.A.Joshi (1988): Flora of melghat Tiger Reserve 1-24.

Dinesh Khedkar and Nitin Atre (2019): Medicinal flora of Melghat for Asthama. Journal of Pharmacognosy and Phytochemistry 8(2):2091-2095.

Sakarkar D. M., U. M. Sakarkar, N. M. Sakarkar, V. N. Shrikhande, J. V. Vyas and R. S.Kale (2010): Medicinal plants used by the tribes for hair disorders in Melghat forest of Amravati District Maharashtra.

Kokate U. R. and G. D. Muratkar; (2012-13): Diversity of Medicinal plants from Melghat Forest.

NOXIOUS WEEDS SPECIES OF HARDA (MADHYA PRADESH), INDIA

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Abstract:

Intensive floristic survey has been carried out in different seasons from 2011 to 2016 by well-plannedschedule. A study was conducted to analyze noxious weeds species of Harda district of MadhyaPradesh. Harda district is located in south western part of Madhya Pradesh.Present study records 902 wild and naturalized species of flowering plants which are distributed in 532 genera and 117 families. Out of 902 species 104 species are noxious weeds species of Harda district which are distributed in 86 genera and 29 families. Herbs are 95 and 9 climbers species of noxious weeds are recorded. *Xanthium strumarium* L.is growing on all type of bushes in soybean it is badly affected. In wheat crop *Avena sps., cyperus sps., steria sps etc*.were found major weeds affecting wheat production.In maize crop *commelina sps. Convolvulus sps. Digitaria sps.* were recorded as highly problematic weeds. Poaceae is most dominant family of the area. Some species is used by tribal as ethno medicine and some are used as vegetablelike *chenopodium alnum* etc. A*vena* is used as fodder grass. Some are used as packing material.These are naturalized in study area which accounts 16 % of total flora. Invasive plants in study area are *Argemone mexicana L.; Ocimum americanum L.; Parthenium hysterophorus L. etc.*

Keywords: Harda, Weeds, Narmada River, Satpura ranges, Malwa plateau.

Introduction:

Harda district is situated in the eastern part of Madhya Pradesh. Harda has been declared as a separate district by Government of Madhya Pradesh on 6th July, 1998. Earlier it was a part of Hoshangabad district. It lies in 21˚ 53` to 22˚36` longitude and 76˚47` to 77˚20`latitude. Total area of the district is 3,334 Km^2. of which forest covers 780.92 Sq. Km. It is bounded by Satpura ranges in the north and southern part of the district is covered by Satpura and extension of Malwa plateau. Population of the Harda district is 570465. The study area is mainly drained by Narmada River and its tributaries are Ganjal, Anjal, Sukni, Midkul, Dedra Machak, Syani, and Kalimachak rivers. The normal rainfall of Harda district is 1124.2 mm. The normal annual mean maximum temperature 32.8˚C and minimum temperature is 19.8˚C. Noxious weeds mean harmful weeds which adversely affect crops productivity and causes health hazards and low production. It affected agriculture productivity. A plant growing at wrong place or when it is not

desired, therefore, may be awed. Weeds are an unwanted plant which is growing in crops and make competition for light, mineral etc. and play role to energy drains in managed ecosystem.

Noxious means harmful weeds that adversely affect crop productivity and cause health hazards in human and animal. It is differ from other weeds in the begging more aggressive that make them more competitive and stabilized in short time. Weed decrease yields of the crop.it casing effect like skin infection and many types allergies. Indirectly it compete with parasitize men utilizing at least part of the energy which in their absence might have become available to men through food chain. They decrease quality of proceed resulting have loss to farmers. Keeping this in mind this study is carried out to explore noxious weed because there is no work is done in this area. A small work is done in this area by Sainkhediya and Ray (2014), Sainkhediya (2015), Sainkhediya (2016) etc.

Material and Methods:

Intensive floristic survey has been carried out in different seasons from 2011 to 2016 by well-planned schedule, covering various vegetation types, altitudinal ranges, wastelands, and weeds of cultivated fields and all other conceivable ecological niches to collected weed species. Field numbers are given to the specimens and data about weed were recorded. For plant collection and preservation of voucher specimens standard methodology has been followed (Jain and Rao, 1977). Voucher specimen were collected in polybag and identified in the laboratory with the help of flora (Hooker, 1892-1897; Cook,1903; Gamble 1915; Haines, 1921-1924; Verma *et al.,* 1994; Mudgal *et al.,* 1997, Duthie 1960, Singh *et al.,* 2001; Khanna *et al.,* 2001) and other available literature were also consulted for identification of plants. Information was collected form farmers. A questionnaire was constructed and surveys were carried out in every village. Photographs have been taken using digital camera. Recent up-to-date nomenclature of ICBN was followed. For changed name of families or botanical names latest classification of APG-III has been followed. Herbarium specimens were deposited in PMB Gujarati Science College, Indore.

Results and Discussion:

Analysis of any flora depends upon the understanding of its geographical elements, which helps in correct interpretations of the flora. Present study records 902 wild and naturalized species of flowering plants which are distributed in 532 genera and 117 families in Harda district of Madhya Pradesh. Out of 902 species 104 species are noxious weeds species of Harda districtwhich are distributed in 86 genera and 29 families. Herbs are 95 and 9 climbers species of

noxious weeds are recorded. Phytogeographical elements of Harda district have been analyzed showed that 33.11 % species of this floristic element are native to India and adjoining areas. Poaceae is the most dominant family in the study area. An analysis of life form shows that herbaceous plantsexhibit maximum diversity. The relatively higher percentage of herbs may be due to predominant weed flora as well as low percentage of trees. *Xanthium strumarium* L.is growing on all type of bushes in soybean it is badly affected. In wheat crop *Avena sps.,cyperus sps.,steria sps etc.* were found major weeds affecting wheat production. In maize crop *commelina sps. Convolvulus sps. Digitaria sps.* were recorded as highly problematic weeds. Poaceae is most dominant family of the area. Some species is used by tribal as ethno medicine and some are used as vegetable like *chenopodium alnum* etc. A*vena* is used as fodder grass. Some are used as packing material.These are naturalized in study area which accounts 16 % of total flora. Invasive plants in study area are *Argemone mexicana L.; Ocimum americanum L.; Parthenium hysterophorus L. etc.*

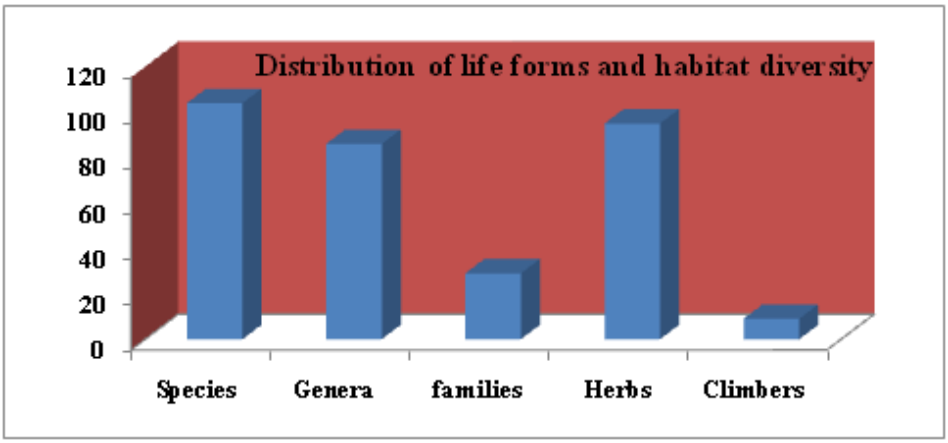

Figure 1: Distribution of life forms and habitat diversity

Conclusion:

The study documented the noxious weeds species of Harda district. This knowledge will be beneficial to explore plant utility in future along with save income of the formers. Health hazard to animal and humans form noxious weeds are many. Some of them are useful weed e.g. *Avena sp.*is used for fodder. Some of them are also medicinally used as well as economically used like that *cynodon dactylon, cyperus rotundus, artimisia sp.* some of them are used as vegetable e.g. *chenopodium album*. Phytogeographical elements of Harda district have been analyzed showed that 33.11 % species of this floristic element are native to India and adjoining areas. The relatively higher percentage of herbs may be due to predominant weed flora as well as low percentage of trees. 145 exotic plant species have been recorded in Harda district it is showed that rich biodiversity of the area.

Table 1: List of noxious weeds species of Harda district

Sr. No.	Family	Botanical name	Habit	Crops		
				Wheat	**Maize**	**Soyabean**
1.	Menispermaceae	*Cocculus hirsutus (L.) Theob.*	C	-	+	+
2.	Papaveraceae	*Argemone Mexicana L.*	H	+	-	+
3.	Cleomaceae	*Cleome gynandra L.*	H	-	+	+
4.		*Cleome viscosa L.*	H	+	-	+
5.	Polygalaceae	*Polygala arvensis Willd.*	H	-	+	+
6.	Portulacaceae	*Portulaca oleraceae L.*	H	-	+	+
7.		*Portulaca quadrifida L.*	H	-	+	+
8.	Malvaceae	*Abelmoschus crinitus Wall.*	H	-	+	+
9.		*Abelmoschus ficulneus (L.) Wight & Arn.*	H	-	+	+
10.		*Abutilon indicum (L.) Sweet*	H	-	+	+
11.		*Corchorus aestuans L.*	H	-	+	+
12.		*Malachra capitata (L.) L.*	H	-	+	+
13.		*Malvastrum coromandelianum (L.) Garcke*	H	+	-	+
14.		*Melochia corchorifolia L.*	H	+	-	+
15.		*Sida acuta Burm. F.*	H	+	-	+
16.		*Sida cordata (Burm.f.) Borss.Waalk.*	H	+	-	+
17.		*Sida cordifolia L.*	H	+	-	+
18.		*Sida rhombifolia L*	H	+	-	+

19.	Zygophyllaceae	*Tribulus terrestris L.*	H	+	-	+
20.	Oxalidaceae	*Biophytum reinwardtii (Zucc.) Klotzsch.*	H	+	-	+
21.		*Biophytum sensitivum (L.) DC.*	H	+	-	+
22.		*Oxalis corniculata L.*	H	+	-	+
23.	Leguminosae	*Alysicarpus pubescens J.S.Law*	H	-	+	+
24.		*Cajanus scarabaeoides (L.) Thou.*	H	-	+	+
25.		*Indigofera linifolia (L.f.) Retz.*	H	-	+	+
26.		*Mimosa pudica L.*	H	-	+	+
27.		*Rhynchosia bracteata Baker*	H	-	+	+
28.		*Rhynchosia minima (L.) DC.*	C	-	+	+
29.		*Tephrosia pumila (Lam.) Pers.*	H	-	+	+
30.		*Vigna trilobata (L.) Verdc.*	H	-	+	+
31.		*Zornia gibbosa Span.*	H	+	-	+
32.	Onagraceae	*Ludwigia octovalvis (Jacq.) Raven*	H	-	+	+
33.	Cucurbitaceae	*Citrullus colocynthis (L.) Schrad.*	H	-	+	+
34.		*Coccinia grandis (L.) Voigt*	C	-	+	+
35.		*Corallocarpus conocarpus (Dalzell & Gibson) Hook.f.*	C	-	+	+
36.		*Corallocarpus epigaeus (Rottler) Hook.f.*	C	-	+	+
37.		*Ctenolepis garcini (L.) Clarke*	C	-	+	-
38.		*Diplocyclos palmatus (L.) C.Jeffrey*	C	-	+	+
39.	Apiaceae	*Centella asiatica (L.) Urb.*	H	+	-	-
40.	Compositae	*Acanthospermum hispidum DC.*	H	-	+	+

41.		*Ageratum conyzoides (L.) L.*	H	+	+	-
42.		*Blumea fistulosa (Roxb.) Kurz*	H	-	+	-
43.		*Cichorium intybus L.*	H	-	+	+
44.		*Conyza japonica (Thunb.) Less. ex Less.*	H	+	-	+
45.		*Eclipta prostrata (L.) L.*	H	+	-	-
46.		*Elephantopus scaber L.*	H	-	+	+
47.		*Emilia sonchifolia (L.) DC. ex DC.*	H	-	+	-
48.		*Lagascea mollis Cav.*	H	-	+	-
49.		*Parthenium hysterophorus L.*	H	+	-	+
50.		*Sonchus asper (L.) Hill*	H	+	-	+
51.		*Tridax procumbens (L.) L.*	H	+	-	-
52.		*Xanthium strumarium L.*	H	+	-	+
53.	Primulaceae	*Anagallis arvensis L.*	H	+	-	-
54.	Loganiaceae	*Spigelia anthelmia L.*	H	-	+	+
55.	Gentianaceae	*Canscora diffusa (Vahl) R.Br. ex Roem. & Schult.*	H	+	+	+
56.		*Centaurium pulchellum (Sw.) Druce*	H	+	+	-
57.		*Enicostema axillare (Poir. ex Lam.) A.Raynal*	H	+	+	-
58.		*Exacum pedunculatum L.*	H	+	+	-
59.		*Exacum tetragonum Roxb.*	H	+	-	+
60.		*Hoppea dichotoma Willd.*	H	+	-	+
61.	Boraginaceae	*Trichodesma zeylanicum (Burm.f.) R.Br.*	H	+	-	+

62.	Convolvulaceae	*Convolvulus arvensis L.*	H	+	-	+
63.		*Convolvulus prostratus Forssk.*	H	-	+	-
64.		*Ipomoea hederifolia L.*	C	-	+	-
65.		*Ipomoea pes-tigridis L.*	C	-	+	-
66.	Solanaceae	*Physalis minima L.*	H	+	-	-
67.		*Solanum virginianum L.*	H	-	+	-
68.	Plantaginaceae	*Stemodia viscosa Roxb.*	H	-	+	-
69.		*Veronica anagallis-aquatica L.*	H	+	-	+
70.	Martyniaceae	*Martynia annua L.*	H	+	-	+
71.	Acanthaceae	*Andrographis paniculata (Burm.f.) Nees*	H	+	-	+
72.		*Rungia pectinata (L.) Nees*	H	+	-	+
73.		*Rungia repens (L.) Nees*	H	+	-	+
74.	Lamiaceae	*Leucas aspera (Willd.) Link*	H	+	-	+
75.		*Leucas biflora (Vahl) Sm.*	H	+	-	+
76.		*Ocimum americanum L.*	H	+	-	+
77.		*Ocimum basilicum L.*	H	+	-	+
78.	Nyctaginaceae	*Boerhavia diffusa L.*	H	+	-	-
79.	Amaranthaceae	*Achyranthes aspera L.*	H	+	-	-
80.		*Alternanthera pungens Kunth*	H	+	-	+
81.		*Alternanthera sessilis (L.) R.Br. ex DC.*	H	+	-	+
82.		*Celosia argentea L.*	H	+	-	+
83.		*Chenopodium murale L.*	H	+	-	+

84.	Euphorbiaceae	*Euphorbia hirta L.*	H	+	-	+
85.	Commelinaceae	*Commelina benghalensis L.*	H	+	-	+
86.		*Cyanotis fasciculata (BHeyne ex Roth) Schult. & Schult.f.*	H	+	-	+
87.	Cyperaceae	*Cyperus compressus L.*	H	+	-	+
88.		*Cyperus corymbosus Rottb.*	H	+	-	+
89.		*Cyperus imbricatus Retz.*	H	+	+	+
90.		*Cyperus iria L*	H	+	+	+
91.	Poaceae	*Avena sterilis L.*	H	+	+	+
92.		*Brachiaria ramosa (L.) Stapf*	H	-	+	+
93.		*Cenchrus ciliaris L.*	H	-	+	+
94.		*Cynodon dactylon (L.) Pers.*	H	+	+	+
95.		*Dactyloctenium aegyptium (L.) Willd.*	H	+	+	+
96.		*Digitaria ciliaris (Retz.) Koeler*	H	+	+	-
97.		*Echinochloa colona (L.) Link*	H	+	+	-
98.		*Eragrostis ciliaris (L.) R.Br.*	H	+	+	-
99.		*Heteropogon contortus (L.) Beauv. ex Roem. & Schult.*	H	+	+	-
100.		*Setaria intermedia Roem. & Schult.*	H	+	+	+
101.		*Sporobolus coromandelianus (Retz.) Kunth*	H	+	+	+
102.		*Sporobolus indicus (L.) R.Br.*	H	+	+	+
103.		*Themeda laxa (Andersson) A.Camus*	H	+	+	+
104.		*Urochloa panicoides P.Beauv.*	H	+	+	+

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References:

Cook (1903): Flora of the presidency of Bombay. Vol. 1-3. BSI Pub. Calcutta, India.

Duthie (1960): Flora of the upper gangetic plains. Vol.2. BSI Publications Calcutta, India.

Gamble (1915): Flora of the presidency of Madras. Vol.1-3. BSI Pub. Calcutta, India.

Haines (1921-1924): The Botany of Bihar and Orissa.Vol.1-6.BSI Reprint, Calcutta, India.

Hooker J. D. (1892-1897): Flora of British India. Vol. 1-7. BSI Pub., Calcutta, India.

Jain and Rao A. (1977): Handbook of field and herbarium method, today and tomorrows.

Khanna K.K., Kumar A., Dixit R.D. and Singh N.P. (2001): Supplementary flora of M. P. BSI Pub.

Mudgal V., Khanna K.K. and Hajara P.K.(1997): Flora of M. P., Vol. 2. BSI Pub. Cal.India.

Sainkhediya and Ray (2014): New addition to the flora of M.P. from Harda, India. Life Sc. Leaf. 37-41.

Sainkhediya, J. (2015): Ethnomedicinally importance vegetable family Cucurbitaceae in Harda district of Madhya Pradesh,India. Res. J. of multiple disciplines 1: 1.PP.11-15.

Sainkhediya, J. (2016): Invasive alien flora of Harda district of Madhya Pradesh. IJAR.6:4.P.P. 343-346.

Singh N.P., Khanna K.K., Mudgal V. and Dixit R.D. (2001): Flora of M. P. Vol.3. BSI Pub. Cal.India.

Verma D.M., Balakrishnan N.P. and Dixit R.D. (1994): Flora of M.P.Vol.1 BSI Pub. Cal. India

NUTRACEUTICALS

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Dietary supplements are substances that are considered foods or their constituents, and in addition to their normal nutritional value, they have health benefits such as disease prevention and health promotion. Due to the side effects of the drug, consumers prefer dietary supplements to improve their health. This has revolutionized dietary supplements around the world. Dietary supplements provide additional health benefits in addition to the basic nutritional value found in foods. Depending on the jurisdiction, the product may claim to prevent illness, improve health, delay the aging process, prolong life expectancy, or support body structure or function. The term dietary supplement includes nutrition and pharmacies and was invented by the doctor in 1989. Stephen L. Defelice, Founder and Chairman of the Foundation for Medical Innovation in New Jersey, USA, offers additional health benefits along with the nutritional value found in foods. These products contain carbohydrates, fats, proteins, vitamins, minerals and other necessary nutrients.

St. John's Wort is used to relieve stress and depression and also acts as a stimulant for tea, coffee and chocolate. Botanical supplements are usually taken in the form of irritating or relaxing tea. Functional foods are another form of dietary supplement. Functional foods are fortified with nutrients and consumed normally, instead of consuming dietary supplements. The two categories of functional foods are: 1) processed foods 2) fermented foods.

Research envisaged:

About bioactive foods that have both health and medicinal properties, such as prevention and treatment of diseases. The need for Chinese herbs is increasing day by day. In a modernized, competitive and stressful environment, all kinds of illnesses have a wonderful day. Most illnesses have symptomatic treatments, but they are not cheap and have a variety of side effects. As a result, many people are prone to lifestyle changes and the use of herbal products. The purpose of this article is to investigate and discuss the effects of dietary supplements on health

care based on disease-specific indications. Development of new nutritional supplements / functional foods / nutritional supplements with new health benefits, health issues related to the need for proper nutrition, non-compliance with known healthy dietary models, these explained the elucidation, definition and understanding of the mechanism of action of products. Aspects of nutritional supplement analysis, formulation and regulation. This article will serve as a tool for getting the latest information.

Various scienctists describes skin aging that is continuously affected by a variety of internal and external factors, including cell biological progression, ultraviolet (UV) radiation, tobacco, nutritional deficiencies, and hormonal imbalances that lead to the destruction of skin cells. Did. By breaking down skin cells, free radicals and inflammation weaken the repair mechanism and cause the breakdown of collagen and elastic fibers. Dietary supplements are of great interest because they are expected to be safe. In this article, new nutritional supplements / functional foods / nutritional supplements with the need for proper nutrition, health issues associated with violations of known healthy dietary models, and new health benefits. Focuses on food development, elucidating the mechanism of action of these products, defining and understanding aspects of nutritional supplement analysis, formulation and regulation. Sumeet Gupta, *et al.,* revealed about The use of foods to promote health and cure illness is well known. Today, most of the drug molecules available in the formulation were used in their raw form in ancient times. Dr. Stephen de Felice first coined the term dietary supplement in 1989 to provide medicinal or health benefits, including the prevention and treatment of illness. This review categorizes a large number of dietary supplements from different sources and their importance. It also discusses the regulatory status of dietary supplements and the latest developments in neutral genomics.

Dietary supplements have multiple therapeutic effects that are not desirable and therefore generate more consumer interest. Increasing shifts to preventative therapies and increasing disposable income, favorable pricing environment, growth in drug retail chains, and increased health care costs are primarily responsible for the growth of the Indian dietary supplement market.Providing information about the use of herbs and supplements alone or with medications may increase the risk of adverse events for the patient. This review aims to assess documented HDS drug interactions and contraindications.

Scope of Nutraceutical:

Health benefits of Nutraceuticals:

From the consumers' point of view nutraceuticals offer following benefits:

- Increased dietary health value.
- Helps you live longer.
- Helps prevent certain illnesses.
- Doing something for yourself can be psychologically beneficial.
- It is more "natural" than traditional medicine and can be considered less likely to cause unpleasant side effects..
- Can represent foods for people with special needs (eg nutritious foods for the elderly)
- Herbal medicine constitutes an effective source of both traditional and modern medicines. According to the World Health Organization (WHO), 80 per cent of rural population depends on herbal medicine for their primary healthcare. Medicinal plants though they are inadequately explored, they provide rich source of a variety of health benefits.

Health Benefits of Phytochemicals:

The health benefits of phytochemicals are

- Substrate for biochemical reactions.
- Cofactors of enzymatic reactions.
- Inhibitors of enzymatic reactions.
- Absorbents that bind to and eliminate undesirable constituent in the intestine.
- Scavengers of reactive or toxic chemicals.
- Enhance the absorption and / or stability of essential nutrients.
- Selective growth factor for beneficial bacteria.
- Fermentation substrate for beneficial bacteria.

Physiological Benefits of Nutraceuticals:

The physiological benefits of nutraceuticals are as follows:

- Cardiovascular agents,
- Anticancer agents,
- Anti-diabetics,
- Anti-obese agents,
- Chronic inflammatory disorders,
- Immune boosters, and
- Antioxidants.

Nutraceuticals and Diseases:

1. Cardiovascular diseases: Cardio-Vascular Diseases (CVD) is a group of disorders of theheart and blood vessels that include hypertension (high blood pressure), coronary heart disease (heart attack), cerebrovascular disease (stroke), heart failure, peripheral vascular disease, etc. It is reported that low intake of fruits and vegetables is associated with a high mortality in CVD. Nutraceuticals in the form of antioxidants, dietary fibers, omega-3 PolyUnsaturated Fatty Acids (ω-3 PUFAs), vitamins, and minerals are recommended together with physical exercise for prevention and treatment of CVD. It has been demonstrated that the molecules like polyphenols present in grapes and in wine alter cellular metabolism and signaling, which is consistent with reducing arterial disease.

Nutraceuticals, vitamins, antioxidants, minerals, weight reduction, exercise, cessation of smoking and restriction of alcohol & caffeine plus other lifestyle modifications can prevent, delay the onset, reduce the severity, treat, and control hypertension. Nutrients and nutraceuticals with calcium channel blocking activity (thus antihypertensive activity) include α-lipoic acid, magnesium, Vitamin B6 (pyridoxine), Vitamin C, N-acetyl cysteine, Hawthorne, Celery, ω-3 fatty acids, etc. Flavonoids, present in onion, endives, cruciferous vegetables, black grapes, red wine, grapefruits, apples, cherries and berries, play a major rolein curing CVD. Flavonoids block the Angiotensin-Converting Enzyme (ACE) that raises blood pressure (BP).

2. Cancer: Cancer has emerged as a major public health problem in developing countries, matching the industrialized nations. A healthy lifestyle and diet can help in preventing cancer. People who consume large amounts of lutein rich foods such as chicken eggs, spinach, tomatoes, oranges and leafy greens experienced the lowest incidence of colon cancer. Chronic inflammation is associated with a high cancer risk. At the molecular level, free radicals and aldehydes, produced during chronic inflammation, can induce deleterious gene mutation and post-translational modifications of key cancer-related proteins. Chronic inflammation is also associated with immune suppression, which is a risk factor for cancer.

3. Diabetes: Diabetes mellitus is characterized by abnormally high levels of blood glucose, either due to insufficient insulin production, or due to its ineffectiveness. The most common forms of diabetes are type I diabetes (5%), an autoimmune disorder; and type 2 diabetes (95%), which is associated with obesity. Gestational diabetes occurs in pregnancy Diabetes, like most chronic health conditions, not only places substantial economic burdens on societyas a whole but also imposes considerable economic burdens on individual patients and their families. Diet therapy is the cornerstone for the management of gestational diabetes mellitus. The uses of herbal dietary supplements are believed to benefit type 2 diabetes mellitus; however, few have

been proven to do so in properly designed randomized trials.

Classification:

Classification of Nutraceuticals Based Upon Food Source:

Food Source	Examples
Plants	β-glucan, Ascorbic acid, γ-tocotrienol, Quercitin, Luteolin, Cellulose, Leutin , Gallic acid, Perillyl alcohol, Indole-3-carbonol, Pectin, Daidzein, Glutathione, Potassium, Allicin, δ-limonene, Genestein, Licopene, Hemicellulose, Lignin, Capsaicin, Geraniol, β-ionone, α-tocopherol, β-carotene, Nor dihydro capsaicin, Selenium, Zeaxanthin, Minerals.
Animals	Conjugated Linoleic Acid (CLA), Eicosa Pentaenoic Acid (EPA), Docosa Hexenoic Acid (DHA), Spingo lipids, Choline, Lecithin, Calcium, Coenzyme Q10, Selenium, Zinc, Creatinine, Minerals.
Microbes	Saccharomyces boulardii (yeast), Bifidobacterium bifidum, Bifidobacterium longum, Bifidobacterium infantis, Lactobacillus acidophilus (LCI), L. acidophilus (NCFB 1748), Streptococcus salvarius.

Immune boosters:

Nutrients present in the diet play a crucial role in maintaining an "optimal" immune response, on the organism's immune status and susceptibility to a variety of disease conditions. A wide range of phytopharmaceuticals with a claimed hormonal activity, called "phytoestrogens", is recommended for prevention of various hormonal imbalance diseases. In this respect, there is a renewed interest in soy isoflavones (genistein, daidzein, biochanin) as potential superior alternatives to the synthetic Selective Estrogen Receptor Modulators (SERMs), which are currently applied in hormone replacement therapy. Phytochemicals integrate hormonal ligand activities and interfere with signaling cascades; their therapeutic use may not be restricted to hormonal ailments only, but may have applications in cancer chemoprevention and / or certain inflammatory disorders as well.

Nutraceuticals that belong to the category of immune boosters and / or antiviral agents are usefulto improve immune function and accelerate wound healing. They include extracts from the coneflowers, or herbs of the genus Echinacea, such as Echinacea purpurea , Echinacea angustfolia Echinacea pillida, and mixtures thereof; extracts from herbs of the genus Sambuca, such as elderberries; and Goldenseal extracts. The coneflowers root extract contains varying amounts of unsaturated alkyl ketones or iso butyl amides. Goldenseal is an immune booster with

antibiotic activity, and includes compounds like berberine and hydrastine, which stimulate bile secretion and constrict peripheral blood vessels respectively. Astragalus membranaceous, Astragalus mongolicus, and other herbs of the genus Astragalus are also effective immune boosters in both natural and processed forms. Astragalus stimulates development and transformation of stem cells in the marrow and lymph tissue to active immune cells.

Classification of Nutraceuticals Based Upon Mechanism of Action

Anticancer	**Positive Influence on Blood Lipid Profile**	**Antioxidant Activity**	**Anti-Inflammatory**	**Osteogenetic (Or)Bone Protective**
Capsaicin Genestein Daidzein α-tocopherol γ-tocotrienol Conjugated Linoleic acid Lactobacillus acidophilus Sphingolipids Limonene Diallyl sulphide Ajoene α-tocotrienol	β-glucan γ-tocotrienol α-tocotrienol MUFA Quercetin ω-3 PUFA Reseveratrol Tannins β-sitosterol Saponins Guar	CLA Ascorbic acid β-carotene Polyphenolics Tocopherols Tocotrienols Indole- 3-carbonol α-tocopherol Ellagic acid Lycopene Lutein Glutathione Hydroxy tyrosine	Linolenic acid EPA DHA GLA Capsaicin Quercetin Curcumin	CLA Soy protein Genestein Daidzein Calcium Casein phosphopeptides FOS (Fructo-Oligo Saccharides) Inulin

Nutraceuticals for Diabeties:

Various forms of dietary supplements / diabetic patients are fortified beverages, fortified baked goods, vitamins, food or leaf extracts, superfood seed extracts, powdered dietary supplements and capsules, pills or tablets. These dietary supplements are not intended to be a substitute for diabetics / hypoglycemic drugs, but their continued use may be useful for diabetics. Some of the diabetic dietary supplements are:

Different scientific societies' recommendations on diet nutritional composition in diabetes:

Parameter	**European Diabetes Association, 2004**	**American Diabetes and Nutritionist Associations, 2008**	**Canadian Diabetes Association, 2008**
Proteins	10–20% ICT	15–50% ICT	15–20% ICT (as generalpopulation)
Carbohydrates	45–60% (personalized)	> 130g/day (personalized)	45–60% ICT
Low glycemic index	Yes	Modest benefit	Yes
Sugar	50 g/day, if there is good control	No limits within totalcontent	< 10% ICT
Fiber	> 40 g/day	14 g/1000 kcal (as general population)	25–50 g/day
Saturated fats	< 10% ICT; < 8% if high LDL	< 7% ICT	< 7% ICT
Total fats	< 35% ICT; < 30% if overweight	Personalized	< 35% ICT
Polyunsaturated fats	< 7% ICT	> 2 fish/week	< 10% ICT
Monounsaturated fats	10–20% ICT	No reference	Use instead of saturated
Cholesterol	< 300 mg/day (less if high LDL)	< 200 mg/day	NA
Omega-3 fatty acids	2–3 servings of fish a week	> 2 servings of fish a week	Consume as much fish asplants
Alcohol	< 10 g (women); < 20 g (men)	1 glass/day (women); 2 glasses/day (men)	Limit to 1/day (women) and 2/day (men), with risk of late hypoglycemia

ICT – total calorie intake, LDL – low-density lipoproteins

***Artocarpus Heterophyllus* (Jackfruit):**

Artocarpus heterophyllus lamb. Also known as jackfruit, it contains a flavonoid known for its antioxidant properties, isoquercitrine. A study published in the Scientific World Journal

found that Jackfruit leaf extract significantly reduced fasting blood glucose by reducing oxidative stress on the pancreas and damage to beta cells.

Formulation of diabetic Nutraceutical:

The appropriate diet composition is < 30% total fat, < 10% saturated fats, > 15 g/1000 kcal fiber,half soluble, 45–60% of carbohydrates with a moderate intake of sugar (50 g/day) and protein intake of 15–20% of the total calories a day.

Sweetening agent for diabetic Nutraceuticals:

It is forbidden to consume sugar in the diet of diabetics. Using other high calorie sweets like fructose or polyalcohol does not seem to have any additional benefits. Fructose has a detrimental effect on plasma glucose and is recommended to be taken as an ingredient in natural foods, not as a sweetener. Polyalcohol provides 2 kcal / g, which makes it interesting for low-calorie diets, but high doses, can cause side effects such as diarrhea. There is consensus that calorie-free sweets are not harmful to your health, but there is no evidence to improve glycemic control in the long run.

Types of diabetes Nutraceuticals:

Nutraceutical supplements

Nutraceuticals	**Action**
L-carnitine	Promotion of insulin sensitivity and hypolipidemic actions
α-Lipoic acid	Treatment of diabetic neuropathy and degenerative neuronal disease
Berberine	Hypoglycemic and hypolipidemic actions
ω-3	Anti-arrhythmic effect and decrease of triglycerides

L-carnitine:

L- Carnitine (β-hydroxyγtrimethylaminobutyrate), a natural vitamin-like compound, is an ubiquitous component of mammalian plasma and tissue, predominantly distributed in skeletal muscle and myocardium. Carnitine is supplied through food sources (meat, dairy products, etc.) and biosynthesis from lysine and methionine. Supplemental studies show that L-carnitine increases insulin sensitivity and has a lipid-lowering effect. Lcarnitine transports fatty acids to the matrix for β-oxidation via the inner mitochondrial membrane, detoxifies potentially toxic metabolites, regulates mitochondrial AcylCoA / CoA ratios, and many other important intracellular cells, and fulfills metabolic function.

Conclusion:

Researchers say dietary supplements are perfectly fine because of their natural health benefits, such as reducing the incidence of high blood sugar levels. However, eating a healthy diet and lifestyle is of paramount importance in reducing your risk of diabetes. Dietary supplement research is hampered by a number of factors that limit their generalizability and make recommendations difficult. High-quality studies are useful for revealing different mechanisms of action, optimal doses, and optimal combinations for different patient populations. Mantzioris said: "People need to invest in complete nutrition, including vegetables and fruits, grains, lean meats, fish, eggs, nuts and seeds, and dairy products. However, if you are considering dietary supplements for the treatment or prevention of diabetes, it is always advisable to consult your doctor. There's still a lot to learn about them.

References:

Tucker G. Nutritional Enhancement of Plants. Current Opinion in Biotechnology. 2003; 14: 221-225.

Brower V. Nutraceuticals: Poised for a Healthy Slice of the Healthcare Market? Nat Biotechnol. 1999; 16: 728-731.

Cook N.C. and Samman S. Flavonoids - Chemistry, Metabolism, Cardioprotective Effects, and Dietary Sources. J. Nutritional Biochem. 1996; 7: 66-76.

Dutta P.C., Phytosterols as Functional Food Components and Nutraceuticals, Marcel Dekker, Edinburgh, 2003.

German J.B. and Walzem R.L. The Health Benefits of Wine. Annual Review of Nutrition. 2000;20: 561-593.

Gita C. Functional Food Attributes of ω-3 Polyunsaturated and Conjugated Linoleic Acid Enriched Chicken Eggs. Current Topics in Nutraceutical Research. 2004; 2: 113-121.

Hollman P.C.H., Feskens E.J. and Katan M.B. Tea Flavonols in Cardiovascular Disease and Cancer Epidemiology. Proc Soc Exper. Biol. Med. 1999; 220: 198-202.

Hollman P.C.H., Hertog M.G.L. and Katan M.B. Analysis and Health Effects of Flavonoids, Food Chem. 1996; 57:43-46.

Houston M.C. Nutraceuticals, Vitamins, Antioxidants, and Minerals in the Prevention and Treatment of Hypertension. Progress in Cardiovascular Diseases. 2005; 47: 396-449.

Israel O., Auguster O., Edith O.A. Antioxidant and Antimicrobial Activities of Polyphenols from Ethanomedicinal Plants of Nigeria. Afr. J. Biotech. 2010; 9: 2289-2293.

J.T. Low Intake of Fruits, Berries and Vegetables is Associated with Excess Mortality in Men: The Kuopio Ischaemic Heart Disease Risk Factor (KIHD) Study. J Nutr. 2003; 133: 199-204.

Nkondjock A. and Ghadirian P. Dietary Carotenoids and Risk of Colon Cancer: A Case-Control Study. Int. J. Cancer. 2004; 110: 110-116.

Rissanen T.H., Voutilainen S., Virtanen J.K., Venho B., Vanharanta M., Mursu J. and Salonen

Sidhu K.S. Health Benefits and Potential Risks Related to Consumption of Fish or Fish Oil. Regul Toxicol Pharmacol. 2003; 38: 336-344.

Sirtori C.R. and Galli C. Fatty Acids and the ω-3. Biomedecine and Pharmacotherapy. 2002; 56: 397-406.

Tank Dharti S., Sanket Gandhi, Manoj Shah. Nutraceuticals. Portmanteau of Scienceand Nature. 2010; 5(3).

Temple W.J. and Gladwin K.K. Fruits, Vegetables, and the Prevention of Cancer: Research Challenges. Nutrition. 2003; 19: 467- 470.

Trottier G., Boström P.J., Lawrentschuk N., Fleshner N.E. Nutraceuticals and Prostate Cancer Prevention: A Current Review. Nat Rev Urol. 2010; 7:21-30.

Whitman M. Understanding the Perceived Need for Complementary and Alternative Nutraceuticals: Lifestyle Issues. Clin J Oncol Nurs. 2001; 5: 190-194.

WHO Release (2003b) Available at http://www.who.int/ mediacentre/news/releases/2003/pr27en/downloaded on 9 April 2007.

Willis M.S. and Wians F.H. The Role of Nutrition in Preventing Prostate Cancer. Clin Chim Acta. 2003; 330:57-83.

PLANT DISEASE EPIDEMIC GROWTH AND ANALYSIS

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Abstract:

Disease on plants usually starts out at a low level, a small number of plants affected and a small amount of plant tissue affected, and it becomes of concern to us only when its incidence and severity increases with time. When we look at some examples of plant disease epidemics from the published literature, we not only notice that the incidence or severity starts near zero and then increases dramatically, but we also can discern some distinct patterns of development with time. An epidemic consists of a population of infected individuals in a (generally larger) host population, and the change in diseased individuals over time and space. Before one can understand or compare epidemics, one must first monitor them. For the purpose of epidemic analysis, this involves sampling for disease, and measurement of disease in the sample. Because plant disease involves the interaction of a plant and a pathogen as influenced by the environment, it is natural to consider measurements of plants (e.g., crops, forest trees), pathogens (e.g., spores), and the physical environment (e.g., ambient air temperature) when quantifying epidemics.

Keywords: Disease epidemic, Disease tetrahedron, Epidemic decline, Disease progress curve.

Introduction:

Plant epidemiology deals with interacting populations of host plants and plant pathogens. This field of science is at the interface of microbiology, plant physiology, ecology, environmental physics, genetics, statistics, economics, and applied mathematics. Plant disease epidemics are cyclical phenomena, that is, they consist of repeated cycles of pathogen development in relation to the host and the environment and to some extent human activity and time (Berger, 1981). The inoculum, which might consist of fungal spores, bacterial cells, nematodes, viruses within an aphid vector, or some other propagules of a pathogen, gains entry

into and establishment within the host tissues through the process of infection. The pathogen develops within the host and eventually begins to produce new inoculum, which, in turn, can be dispersed to new susceptible sites to initiate new infections. Pathogens that produce only one cycle of development (one infection cycle) per crop cycle are called monocyclic, while pathogens that produce more than one infection cycle per crop cycle are called polycyclic. Generally in temperate climates there is only one crop cycle per year, so the terms "monocyclic" and "polycyclic" are based on the number of cycles per year. In tropical or subtropical climates, however, there can be more than one crop cycle per year, and it is important to remember that "monocyclic" and "polycyclic" are based on a single crop cycle. These same terms are used to describe the epidemics as well as the pathogens, so we often speak of a "monocyclic epidemic" or a "polycyclic epidemic". For some diseases it is important to consider an epidemic over a period of many growing seasons. This is particularly true for perennial plants (forages, pastures, lawns, orchards, forests, etc.) or for annual crops that are grown in monoculture year after year. In these situations the inoculum produced in one growing season carries over to the next, and there could actually be a buildup of inoculum over a period of years. In the tropics there may not be clear-cut breaks between growing seasons such as we find in the temperate zones, and epidemics can be virtually continuous over periods of many years on such crops as bananas, coffee, and rubber trees. We refer to these epidemics as polyetic epidemics, regardless of whether the pathogen is monocyclic or polycyclic within each season (Berger, 1989).

Epidemic:

- Slow epidemic (Tardive epidemic)
  - Occurs in monocyclic diseases
  - On perennial plants
  - E.g. citrus tristeza; Dutch elm disease
- Fast epidemic (Explosive epidemic)
  - In polycyclic diseases
  - Annual crops
  - E.g. rice blast, potato blight (Campbell, C. L., and Madden, L. V. 1990)

Epidemic and epidemiology:

When a pathogen spreads to and affects many individuals within a population over a relatively large area and within a relatively short time, the phenomenon is called an epidemic (Leonard and Mundt, 1984)

- ✓ An epidemic has been defined as any increase of disease in a population.
- ✓ A similar definition of an epidemic is the dynamics of change in plant disease in time and space.
- ✓ The study of epidemics and factors influencing them is called epidemiology.
- ✓ Epidemiology is concerned simultaneously with populations of pathogens and host plants as they occur in an evolving environment, i.e., the classic disease triangle (Leonard and Mundt, 1984.)

Questions in one's mind during outbreak of disease:

- ✓ What will happen over the next few weeks?
- ✓ Will all the plants die, leaving nothing to harvest? Or
- ✓ Will only currently infected plants yield less?
- ✓ Are all the plants infected and only few showing symptoms?
- ✓ Is the pathogen air/water/wind/vector dispersed?
- ✓ Can this crop be planted next season etc.?

Epidemiology:

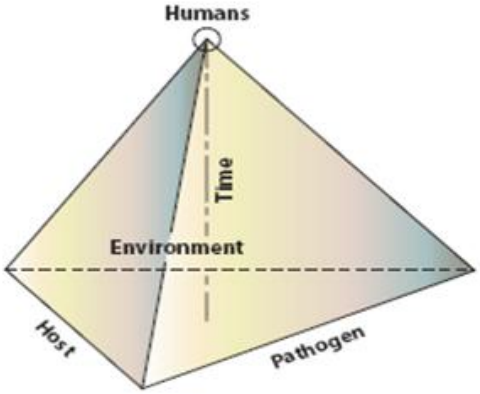
- ✓ Thus epidemiology can be defined as the study population of pathogen in the population of host and the resulting disease under the influence of environmental and human factors
- ✓ Epidemiology helps in answering entire questions by describing disease development pattern during the single season and from year to year (Teng, 1985).

Epidemics of past:

- ✓ The Irish potato famine of 1845–1846 was caused by the Phytophthora late blight epidemic of potato,
- ✓ The Bengal famine of 1943 was caused by the Cochliobolus (Helminthosporium) brown spot epidemic of rice (Vanderplank, 1963).

The disease tetrahedron

- ✓ Susceptible host
- ✓ Virulent pathogen
- ✓ Favourable environment
- ✓ Development of disease is affected by
- ✓ Duration & frequency of each element of over time,
- ✓ Duration & frequency of favourable environment.



Interrelationships of the factors involved in plant disease epidemics (Tjosvold, 2018)

(Source: Encyclopedia of Microbiology (Third Edition), 2009)

The elements of an epidemic:

In fungal & bacterial diseases:

- ✓ The Host
- ✓ The Pathogen
- ✓ Environment
- ✓ Human activity

In virus and virus like diseases

- ✓ The Virus
- ✓ The Host
- ✓ The Vector
- ✓ Environment (Waggoner, 1974)

Factors affecting development of epidemics

1. Host factors

- **Levels of Genetic Resistance or Susceptibility of the Host**

- ✓ Highly resistant
- ✓ Moderately resistant
- ✓ Susceptible

- **Degree of Genetic Uniformity of Host Plants**

- ✓ Monoculture (vertical/ horizontal resistance) e.g.

Cochliobolus (Helminthosporium) blight on Victoria oats and in southern corn leaf blight on corn carrying Texas male-sterile cytoplasm.

- **Type of Crop**

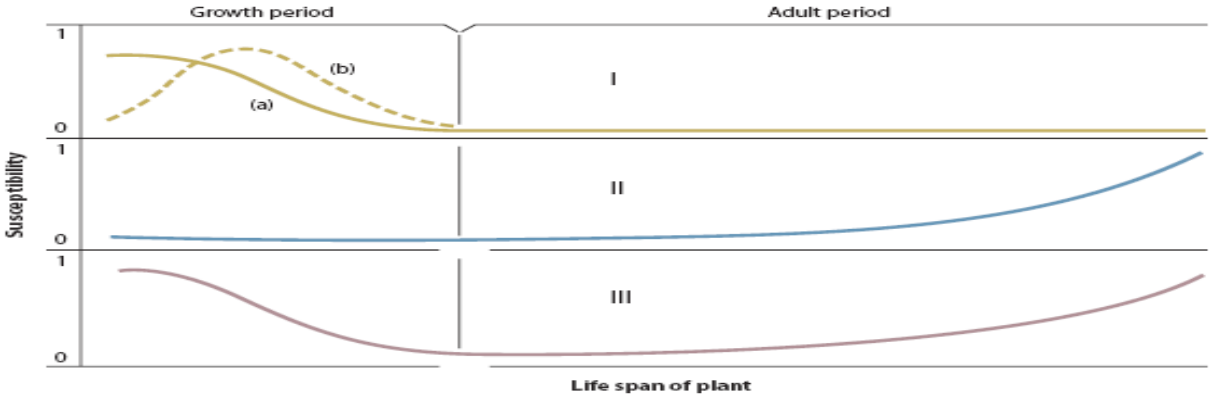
✓ Annual

✓ Perennial

- **Age of Host Plants**

✓ Plants change in their reaction (susceptibility or resistance) to disease with age. The change of resistance with age is known as ontogenic resistance (van den Bosch, 1988).

Change of susceptibility of plant parts with age



(**Source: Department of Plant Pathology, CSK HPKV, Palampur)**

- In pattern I, plants are susceptible only in the stages of maximum growth (Ia) or in the earliest stages of growth (Ib).
- In pattern II, plants are susceptible only after they reach maturity, and susceptibility increases with senescence.
- In pattern III, plants are susceptible while very young and again after they reach maturity (Farber, 2017).

2. Pathogen factors

- Levels of Virulence
- Quantity of Inoculum near Hosts
- Type of Reproduction of the Pathogen

✓ Sexual (oospores, ascospores)

✓ Asexual (conidia, zoospores)

- Ecology of the Pathogen

✓ Ectoparasites

✓ Endoparasites

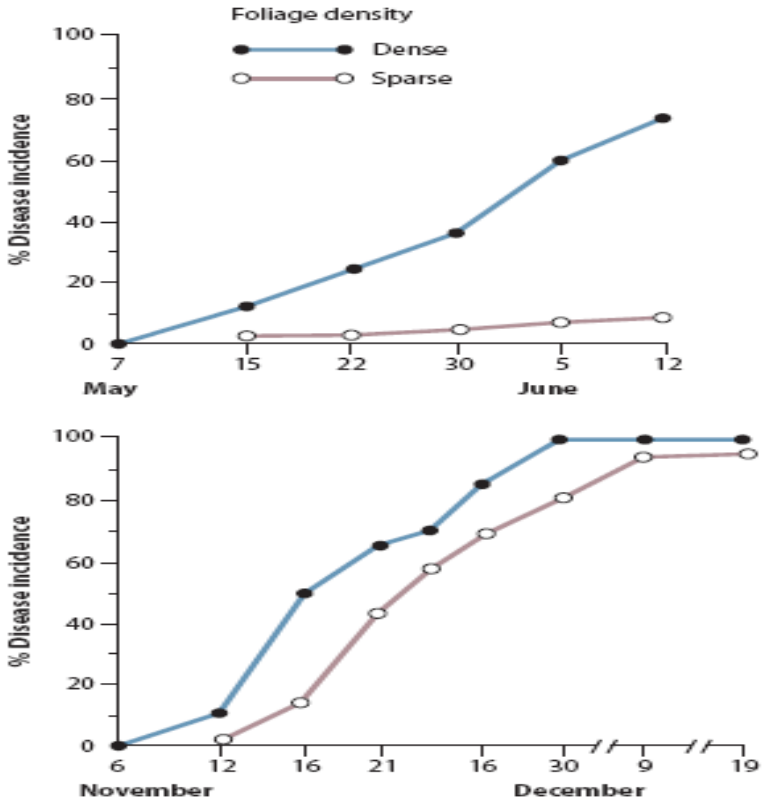
- Mode of Spread of the Pathogen

✓ Active

✓ Passive dispersal (Waggoner, P. E., 1974)

3. Environmental factors

- Moisture
- Temperature



(Source: Department of Plant Pathology, CSK HPKV, Palampur)

Effect of foliage density on development of *Phytophthora infestans* during a period of partly favorable weather (May–June) and of very favorable weather (November– December) (Vanderplank, 1963).

Human activity- A key player in modern epidemics

- Site Selection and Preparation
- Selection of propagative Material
- Cultural Practices
- Disease Control Measures
- Introduction of New Pathogens (Jeger, 2000)

Measurement of plant disease and of yield loss

The disease is measured in term of:

- Disease incidence (disease prevalence)
- Disease severity (extent of damage to host)
- Yield loss (the yield loss is the difference between attainable yield and actual yield) (Vanderplank, 1963)

Epidemic decline

- Saturation of the pathogen in the host population
- Decline of proneness of the host
- Reduction in aggressiveness of the pathogen
- No new infections
- Repeated infections of the host etc (Arneson, 2021).

Patterns of epidemics

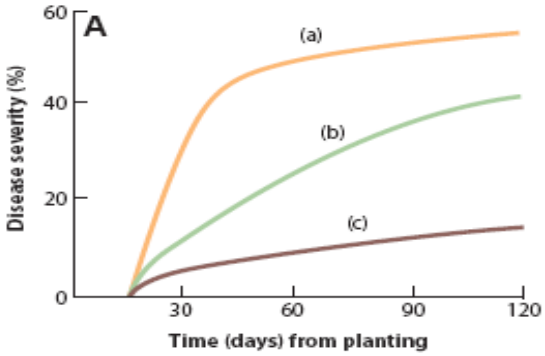
- Interactions among the elements of epidemics, as influenced over time by factors of the environment and by human interference, are expressed in patterns and rates.
- Disease–progress curve
- Disease gradient curve

Disease progress curve

The progress of an epidemic measured in terms of the numbers of lesions/ the amount of diseased tissue, or the numbers of diseased plants plotted over time is called the disease–progress curve (C. A. Gilligan, 1990).

(A) Saturation type of curve

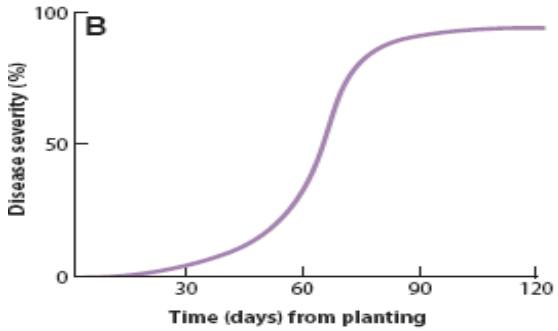
- Three monocyclic diseases of different epidemic rates.



(Source: Directorate of IT, University of Sargodha)

(B)Sigmoidal curve

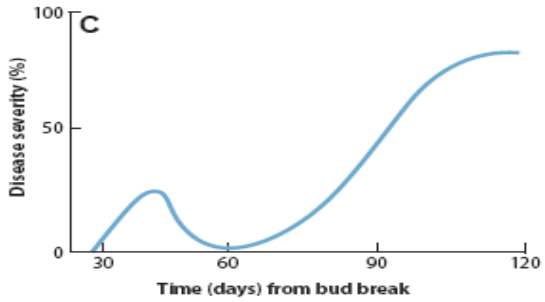
- Polycyclic disease, such as late blight of potato.



(Source: Department of Plant Pathology, CSK HPKV, Palampur)

(C) Bimodal curve

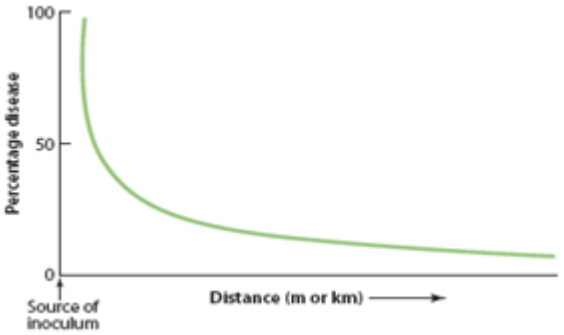
- Polycyclic disease, such as apple scab, in which the blossoms and the fruit are infected at different, separate times.



(Source: Department of Plant Pathology, CSK HPKV, Palampur)

Disease gradient curve

The progress of an epidemic measured in terms of changes in the number of lesions/ the amount of diseased tissue, and the number of diseased plants as it spreads over distance, is called disease gradient curve (spatial pattern).



(Source: Department of Plant Pathology, CSK HPKV, Palampur)

Disease–gradient curve: The percentage of disease and the scale for distance vary with the type of pathogen or its method of dispersal (Esker, 2007).

- ✓ Being small for soil borne pathogens or vectors and
- ✓ Larger for airborne pathogens.

New tools in epidemiology

- Molecular Tools
- Geographic Information System
- Global Positioning System
- Geostatistics
- Remote Sensing
- Image Analysis
- Information Technology

References:

Berger, R.D. 1989. Description and application of some general models for plant disease epidemics. In: Leonard, K.J. and Fry, W.E., (ed), Plant Disease Epidemiology Population Dynamics and Management, Vol. 2. McGraw Hill, New York, pp. 125–149.

Bergot, M., Cloppet, E., P´erarnaud, V., D´eq´ue, M., Marc¸ais, B. and Desprez-Loustau, M.L. 2004. Simulation of potential range expansion of oak disease caused by Phytophthora cinnamom under climate change. Global Change Biology 10: 1539–1552.

Bouma, E. 2007. Computer aids for plant protection, historical perspective and future developments. EPPO Bulletin 37: 247–254.

Bourgeois, G., Bourque, A. and Deaudelin, G. 2004. Modelling the impact of climate change on disease incidence: A bioclimatic challenge. Canadian Journal of Plant Pathology 26: 284–290. the main problem? International Potato Center, Impact on a changing world: Program report, 1997–1998. Lima: CIP

Chan, M.S., Jeger, M.J. 1994. An analytical model of plant virus disease dynamics with rouging and replanting. Journal of Applied Ecology 31: 413–427.

Coakley, S.M., Scherm, H. and Chakraborty, S. 1999. Climate change and plant disease management. Annual Review of Phytopathology 37: 399–426.

Cooke, L.R., Schepers, H.T.A.M., Hermansen, A., Bain, R.A., Bradshaw, N.J., Shaw, D.S., Evenhuis, A., Kessel, G.J.T., Wander, J.G.N., Andersson, B., Hansen, J.G., Hannukkala, A., Nærstad, R. and Nielsen, B.J. 2008. Epidemiology and integrated control of potato late

blight in Europe. In: Peters, R.D., (ed.), Current concepts in potato disease management (In press).

Drenth, A. 2004. Fungal epidemics – does spatial structure matter? New Phytologist 163: 4–7.

Fankhauser, C. 1999. Main diseases affecting seed degeneration in Ecuador: New perspectives for seed production in the Andes. In: European Association for Potato Research. EAPR, Sorrento Italy, pp. 50–1.

Fleming, R.A. and Bruhn, J.A. 1983. The role of mathematical models in plant health management. In: Khommedahl, T. and Williams, P.H. (ed), Challeging Problems in Plant Health. American Phytopathological Society, St. Paul, MN, pp. 368–78.

Gitaitis, R. and Walcott, R. 2007. The epidemiology and management of seedborne bacterial diseases. Annual Review of Phytopathology 45: 371–397.

Hadders, J. 2008. An example of integrated forecasting system for Phytophthora infestans on potato. In: Ciancio, A. and Mukerji, K.G. (ed), Integrated Management of Diseases Caused by Fungi, Phytoplasma and Bacteria. Springer, 179–189.

Hannukkala, A.O., Kaukoranta, T., Lehtinen, A. and Rahkonen, A. 2007. Late-blight epidemics on potato in Finland, 1933–2002; increased and earlier occurrence of epidemics associated with climate change and lack of rotation. Plant Pathology 56: 167–176.

Jeger, M.J. 2000. Theory and plant epidemiology. Plant Pathology 49: 651–658.

Jeger, M.J. 2004. Analysis of disease progress as a basis for evaluating disease management practices. Annual Review of Phytopathology 42: 61–82.

Jeger,M.J., Holt, J., Van Den Bosch, F. andMadden, L.V. 2004. Epidemiology of insect transmitted plant viruses: Modelling disease dynamics and control interventions Physiological Entomology 29: 291–304.

Jeger, M.J. and Pautasso, M. 2008. Plant disease and global change – the importance of long-term data sets doi:10.1111/j.1469-8137.2007.02312.x. New Phytologist 177: 8–11.

HISTOPATHOLOGY OF LEAF GALL OF *SYZYGIUM CUMINI* (L.) SKEELS

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Abstract:

The present investigation deals with the histopathology of leaf gall of *Syzygium cumini* (L.) Skeels induced by Psyllid. Among different types of galls on the leaves of *Syzygium cumini* the gall induced by *Megatrioza vitiensis* (Kirkaldy) is a common occurrence. The leaf galls are epiphyllous, pustaloid, hemispherical, rarely sub globose, sessile, glabrous, greenish yellow. The structure of gall tissue differs from normal leaf in several features. The greatest bulk of the gall is composed of large parenchyma cells, usually closed packed together, with little intercellular spaces and without chlorophyll. The dehiscent pouch gall moderately thick walled with large gall cavity, ostiole minute and hypophyllous. The layer that immediately surrounds the larval cavity consists of thick walled cells. The cells containing tannin are seen in patches surroundings the gall cavity. The gall cavity is surrounded by few secretory cavities.

Keywords: *Syzygium cumini*, Psyllid, larval cavity, epiphyllous, gall cavity and secretory cavity.

Introduction:

Plant galls are abnormal, uncontrolled largely independent growth of the host tissues which arise under the influence of an external stimulus. Development and anatomy of several insect induced galls on certain plant species has been worked out by many workers (Kostoff and Kendall, 1929; Lewis and Walton, 1947; Meyer, 1965; Kant and Arya, 1971; Raman *et al.*, 1978; Kant and Sharma 1981; Ananthakrishnan and Raman, 1988; Kant and Karnawat, 1989; Kant *et al.*, 1994; Ramani *et al.*, 1994). Amerjothy (2005) has reported brief description of leaf gall structure of *Syzygium cumini* induced by an insect of *Megatrioza vitiensis*. This investigation is therefore concerned with the histopathology of the leaf gall produced on *Syzygium cumini* by Psyllid.

Materials and Methods:

Gall and normal leaf material was collected from infested plants growing in Placepalayam reserve forest and adjoining areas and fixed in FAA (Formalin + Acetic acid + Ethyl alcohol 70%). Dehydration, clearing and embedding were done following the tertiary butyl alcohol method as recommended by Johansen (1940). Microtome sections were cut at a thickness of 7-14 µm and stained with Methylene blue. External morphology of the gall was studied under the Stereoscopic microscope.

Observations:

Morphology of the leaf gall

Leaf galls are epiphyllous, pustaloid, hemispherical, subpyriform, rarely sub-globose, sessile, glabrous, greenish yellow (Fig.1.2, 3 & 4) and often with pinkish or brownish, hard, brittle rarely soft and fleshy deciduous and dehiscent pouch gall, generally turning dark brown, and very hard when old; a number of galls often become together into globose irregular mass and cracked in several places by deep fissure and often involving a whole leaf blade (Fig.1.5,6 &7). The average length of the leaf gall ranges from 1.2 -1.6 cm and 6-10 mm in diameter (Fig.1.7)

Anatomy of the normal leaf

The normal leaf is bifacial and xeromorphic. The adaxial and abaxial uniseriate epidermal cells are coated with thick cuticle. The stomata are usually anamocytic type. Mesophyll occupies the vertical rows of palisade tissues, below the adaxial epidermis. Mesophyll contains stone cells. Spongy parenchyma cells are filled with brown tanniniferous contents (Fig.2.1). Main vascular strand apparently bicollateral (Fig.2.2), but adaxial phloem not always very clearly defined. Cells containing tannin abundant in all lignified tissues. A noteworthy feature is the presence of secretory cavities, which are lived with a distinct epithelium when young, and invariably secrete oily substances. The cavities are found below the epidermis on either side of the mesophyll and in this position appear as transparent dots (Fig.2.2).

Anatomy of the gall

The transaction of the galled leaf has an adaxial and abaxial epidermis, is more or less similar to normal leaf and it is coated with thick cuticle. Hairs are absent. Some stomata are evident on the upper epidermis of galled lamina (Fig.2.3). But on the abaxial epidermis, there are a few stomata with anomalies, perhaps due to the galling effect. The type of stomata is anamocytic. The epidermal cells posses highly wavy margins. The inner walls abutting the stomatal aperture are highly thickened by the deposition of the cell wall material. Stomatal number is less, compared to the normal lamina. Mesophyll region is not well differentiated into

palisade and spongy parenchyma tissue. Palisade tissue is reduced compared with the normal leaf. Only one vertical rows of the palisade tissue below the adaxial epidermis; opposite to the gall cavity there is no palisade tissue. The greatest bulk of the gall is composed of large parenchyma cells, usually closed packed together, with little intercellular spaces and without chlorophyll (Fig.2.4).

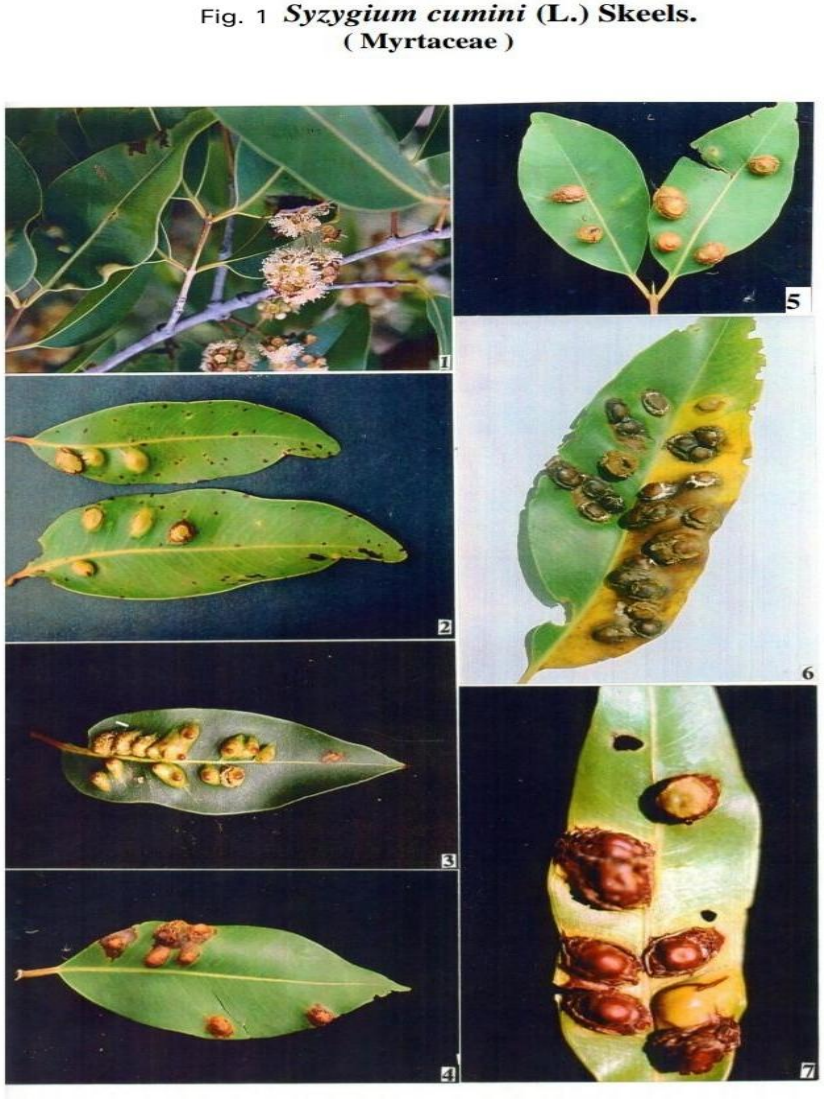

Figure 1.1: A flowering shoots with gall bearing leaves

Figure 1.2: Young elliptic, epiphyllous gall

Figure 1.3: Later stage of above gall

Figure 1.4 and 1.5: Matured galls

Figure 1.6 and 1.7: Breaking of the mature galls through irregular cracks

The dehiscent pouch gall moderately thick walled with large gall cavity; ostiole minute and hypophyllous. The layer that immediately surrounds the larval cavity consists of thick walled cells. There are no erineal hairs in the gall cavity. Cells containing tannin are seen in patches surroundings the gall cavity. Secretory cavities are lined with a distinct epithelium and are more in number on the adaxial epidermis compared with normal leaf; the gall cavity is surrounded by few secretory cavities (Fig.2.5). The vascular bundles occur in a transverse row in the middle of galled parts (Fig.2.6 &7). However, the bundle sheath cells mix with the gall tissue and lose their identity (Fig.2.6).

Fig. 2 ***Syzygium cumini* (L.) Skeels.**

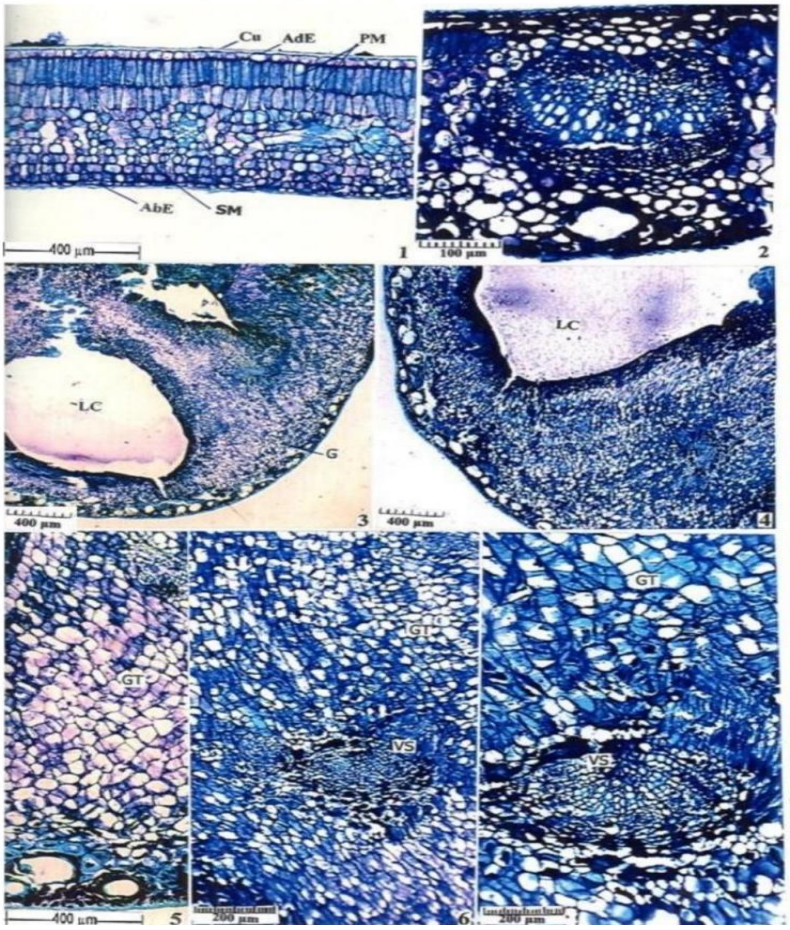

Figure 2.1: Transaction view of normal leaf

Figure 2.2: T.S. of normal leaf, midrib region

Figure 2.3: T.S. of gall through larval cavity

Figure 2.4: A section of above magnified

Figure 2.5: Tissue zones around the gall cavity enclosing secretory cavities

Figure 2.6 and 2.7: Vascular bundles of the gall magnified

Discussion:

The present investigation reveals that major tissue reorganization is brought about by Hypertrophy and Hyperplasia. The cell proliferation is centered in cortical parenchyma and vascular tissues which leads to gall formation. Similar findings have been reported by Klein and Meyer, 1963; Rohfritsch 1971, 1980; Raman and Ananthakrishnan, 1983; Kant and Ramani, 1988; Kant and Kharnawat, 1989; Kant *et al.*, 1994.

Many investigators have suggested that gall formation in plants is due to the stimulus secreted by the gall inducing organism (Norris 1979; Sokhi and Kapil, 1984, 1985; Munuswamy *et al.*, 2019). Incase of *Syzygium cumini* leaf gall therefore it can be suggested that the developing larva provides a stimulus while some how relieves the host tissue from its inbuilt correlation factors. Hypertrophy and Hyperplasia seem to be manifestation of this stimulus and hence play a major role in gall formation.

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References:

Amerjothy S (2005). Plant gall ecology in Scrub ecosystem, MEON, GOING, Project report.

Ananthakrishnan T. N. and Raman.A (1988). Insect plant interaction problem and perspectives in:*Eds dynamics of insect plant interaction* Oxford and IBH Public Co New Delhi 1-1B.

Johansen D. A (1940). *Plant microtechnique.* McGraw and Hill book Co INC N Y London 491.

Kant U and HC Arya (1971). Anatomy of leaf gall on *Salvadora persica* Linn. Induced by *Eriophyes Marcellia.* 37:47-57.

Kant U and H L Sharma (1981). Studies on *Cordia dichotoma* leaf galls induced by EriophyesCordia In: Jain. P Ed. *K Form structure and Function in Plants.* Part 2 Today and Tomorrow Publishers N. D. 181-189.

Kant U and V Ramani (1988). Insect induced galls on certain economically important plants of arid and semiarid regions in: *Dynamics of Insect plant interaction*. (Ananthakrishnan TN and Raman A eds.) Oxford and IBH Public. Co. New Delhi. 165-176.

Kant U and A Karnawat (1989). Histopathological studies on insect induced leaf gall on *Syzygium jambolanum* DC. In Plant science research in India. (Trivedi MLB S Gill and SS Saini eds.) Today and Tomorrow printers and Publishers New Delhi p. 621-625.

Kant U, P Lodha K Sharma and A.K Gupta (1993). Histopathology of leaf gall on *Pongamia glabra* vent. induced by *Eriophyes Cheriani* Massee. Journal of Indian botanical society 73:197-206.

Klein C and Meyer (1963). Etude du development de la galli de *Perriseiagalli*L W sir *Gallium mollugo* L. Marcellia 31:77-94.

Kostoff D and J Kendall (1929). Studies on the structure and development of certain cynipid galls. *Biol. Bull.* 56:402-458.

Lewis I F and L Walton (1947). Initiation of the cane gall of Witch Hazel. Science. 106:419-420.

Mani M. S (1973). Plant galls of India. The Mac Millan Company of India Limited. 354.

Meyer. J (1965). Sur La ValeurMorphologique *et al* development des emergences de la galle de *Rhodites mayri.* Marcellia 32:217-236.

Munuswamy. E, Sivashankari. S and Krishnan. V (2019). An unusual leaf margin role gall of a Psyllid on *Syzygium jambolanum.* DC (Myrtaceae). International journal of Research and Analytical Reviews (IJRAR). 6:2, 751-753.

Norris DM (1979). How insect induced diseases? In: Plant disease: An advanced Treatise (Horsefall JG and EB Cowling eds.) Vol. IV Acad. Press NY 239-255.

Raman A, Ananthakrishnan TN and S. Swaminathan (1978). On the simple leaf galls of *Casearia PS Pstomentosa* Roxb. Induced by Gynaikothrips *Flaviantennatures moutton* (Thysanoptera Phalothripidae). Proc.Ind.Acad.Sci. 87B: 231-242.

Raman A and TN Ananthakrishnan (1983). Studies on some thrips (Thysanoptera: insecta) induced galls 2 Fine structure of the nutritive zone. Pro. Indian. Acad. Sci. B 49:525-561.

Ramani V, P Jain K Sharma and U Kant (1994). Insect induced galls on flowers and seeds. In Vistas in Seed Biology. (Singh T & PC Trivedi eds.) 113-119.

Rohfritsch O (1971). Infrastructure des cellulas du tissue nourricier de la galle de*Geocryptagalli*4-L W sur *Galium mollugo* L. C R Acad. Science. Paris 272:76-78.

Rohfritsch O (1980). Relations has Parasite an debut de la cicidogenise. Social. Bot. Fr. (Actual Bot) 127(1): 199-207.

Sokhi J and RN Kapil (1984). Morphogenetics changes individual by *Trioza* in flowers of *Terminalia arjuna* I Androecium. Phytomorphology 34: 117-128.

Sokhi J and RN Kapil (1985). Morphogenetics changes individual by *Trioza* in flowers of *Terminalia arjuna* II Androecium. Phytomorphology 35: 69-82.

IMPACT OF LOCKDOWN ON ENVIRONMENT

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Abstract:

Since the last century, a disease caused by the infection of Corona virus is the most widely spread and deadliest pandemic that is known as Covid-19. First ever case of corona infection was reported in China in the last week of November 2019. Within the next 75 days, this virus caused havoc all over which resulted in millions of death worldwide. Due to this, lockdown has to be implemented worldwide at various levels in more than 200 countries of the world. As a result, all economic activities came to a halt worldwide. All the industries, power stations and services saw a complete shut down at once since they were established. Similarly, all trains, buses and flights services were closed at once. Shopping malls, markets and trading firms were closed and street sellers, hawkers and deliveries of all kinds stopped their activities. As a result, the world's economy doomed and earning of money was stopped at all stages by everyone. Due to sudden lockdown, lot of people, workers, migrants, tourists and travelers were confined to some or other place with no help or support at all from any source. Hence, it is obvious that the lockdown created huge problems for whole society. However, there are a number of positive effects of lockdown as well. As industries, power stations and construction work producing a lot of heat and pollution and all means of transportation causing huge pollution by burning of fossil fuels came to halt, the production of compounds, emissions, hazardous byproducts and air, water and noise pollution at huge levels also stopped and atmosphere started to become cleaner and cleaner. Within a month, the environment became so clean that the mountains of Dhauladhar ranges in Himachal Pradesh were able to be seen from the city of Jallandhar in Punjab, which is more than 200 kilometers from the mountains. The water of all worst polluted rivers became cleaner than ever. Oxygen level in all rivers has gone up and aquatic animals and plants have got a new life. Reduction in air pollution levels brought relief to persons suffering from anxiety, insomnia, stress and problems related to heart and blood pressure. Similarly, reduction of noise pollution has provided an opportunity for wild animals to intrude into human colonies and areas of human interference. A number of wild animals such as wildebeests, deer, antelopes, bears etc.

were seen roaming on the streets of cities located near forest areas. It seems that nature is refreshing itself by means of what humans find to be a pandemic. It can be said that in this way, nature has shown as the path to restore its freshness and purity. This has caused environmentalists to ponder over shutting all human activities for a few days every year so that the nature could get purified and refreshing in order to keep the earth a healthier place to live for all living beings.

The present study deals with the effects of lockdown on environment and working on ways to keep our environment safe from hazardous human activities.

Keywords: Covid-19, Corona, Pandemic, Lockdown, Environment etc.

Introduction:

Humans have been destroying nature for several centuries, and this destruction has escalated since the industrial revolution. Industries release several dangerous chemical wastes that are causing air pollution and water pollution, including oceans, rivers, and Wildlife. These things were not enough that humans started deforestation to build their houses and buildings and several other things. However, along with deforestation use of people started to use plastic in disturbing quantity. The problem with using plastic is that it is not biodegradable and does not decompose for over 400 years. To make cities look clean, governments started to throw their trash in oceans, causing water pollution. This paper includes the impact of lockdown on air, water, and land pollution and how the lockdown was the worst condition for the world economies. Still, indeed, it was a break for the world from the dangerous gasses, pollution, and chemicals. This paper also critically discusses how lockdown was terrible for the world and helpful for the earth.

Healing of ozone layer

During the lockdown, the ozone layer, which protects the earth from harmful UV rays of the sun, has also been observed to be healed. Due to the reduced air pollution, the air pollutants were also reduced. For the last few years, the ozone layer has been depleting due to the greenhouse gases such as carbon dioxide, methane, and other gases such as chlorofluorocarbons from aerosols, hair sprays, etc. But during the lockdown, due to a slight decrease in air pollution, the ozone layer has surprisingly been healed. This protects our environment from the UV rays. Most of the cases are that the rays from the components used in the refrigerator are proved defective for the ozone layer (Explained Desk, 2020).

The ozone layer has great importance in saving our lives as we live on the surface of the earth and take a safe and healthy breath. This is all because of the ozone layer. The rays coming

from the sun are scorching and not suitable for human skin. They cause hazardous diseases in the human body. This is the reason that the presence of the ozone layer actually secures us. The ozone layer must be present in our atmosphere if we need a healthy life or a life (Explained Desk, 2020). This statement can show the importance of the ozone layer. So, the lockdown is the primary factor of the recovery of the ozone layer as the environmental factors were reducing it and damaging it. So, this lockdown has proved very effective for the ozone layer. When the activities are controlled, the ozone reduction and destruction are also controlled. This is the very major benefit of the lockdown situation.

Positive impact of lockdown on environment

Moving forward, most of these problems were paused for a short time during the lockdown due to Covid-19. During these times, several industries were utterly shut down, and thousands of businesses and millions of people got unemployed globally; however, the earth was suffering significantly, it was stated that CO_2 was increased precisely 1% per year in the previous ten years (Jackson *et al.*, 2019). However, during the lockdown, this trend got completely turned around, and CO_2 emission was reduced about 17% (-11 to-25) by April 7, 2020 (Quere *et al.*, 2020),

Additionally, lockdown practically shut down the world; on the one side, people lost their jobs, houses, and businesses. The other side of the lockdown's impact was entirely positive for the environment as AQI (Air Quality Index) is a measurement tool for air quality within a particular region. If the AQI is lower, for example, below 100, AQI areas are better to breathe; similarly, if the AQI is higher than 100 than that it is unhealthy and crossing 200 or more, it becomes deadly to breathe in that particular environment. Out of the top 40 most polluted cities, there are 32 cities from India, showing the impact of global warming and climate change on India. However, Zambrano-Monserrate *et al.* (2020) reported that, during the lockdown, AQI from the northern, southern, eastern, western, and central regions of India was decreased by 44%, 33%, 29%, 32%, and 15%, respectively, which was a significant improvement in the Air Quality of India (Arora *et al*., 2020).

Impact on water pollution

Covid19 increases the awareness of human's bad habits, and everyone has acknowledged the danger civilized on uncivilized societies are portraying to every living breathing being in the world, and aquatic life is no different. Lockdown during 2020 and 2021 was breathing time for the canals, rivers, and ocean life. According to (Gatehouse, 2019), there will be more trash in the

ocean than there are fishes in the seas. Currently, there are 150 million tonnes of plastic in the mighty ocean, 'the house of sea creatures' (Edmond, 2020).

The use of plastic reduced significantly during the lockdown, and it was supposed to be a happy time for the ocean and sea life; however, coronavirus waste, including single-use personal protective equipment (PPE), started flooding the vast seas. Lockdown or the global pandemic should have seen much-reduced use of plastic materials because industries were shut down who used plastic. In contrast, the use of plastic increased in 2020 because of PPE, the use of packet foods, the plastic used to deliver food at home. In the UK, masks and gloves used by everyone reached the ocean were several million per day. Considering everyone used a mask a day that were billions of masks used by masses to protect themselves from this disease (Edmond, 2020).

Additionally, Charlotte Edmond, 2020, stated that "Waterlogged masks, gloves, hand sanitizer bottles, and other coronavirus waste are already being found on our seabed's and washed up on our beaches, joining the day-to-day detritus in our ocean ecosystems" (Edmond, 2020). One study calculated that contaminated waste thrown in the ocean in the UK was increased by 66,000 tonnes due to a single mask used by each person in the UK and 57,000 tonnes of plastic packaging. Comparing the population gap between the UK and the rest of the world, humans make it even difficult for aquatic life to breathe into clean water (Edmond, 2020).

Impact on the aquatic environment

When we talk about the environment, we also have to include the aquatic environment. This is the environment where the fish and other aquatic animals live. This is a very important environment for us. We are basically attached to this environment. We get some parts of the food from this environment too. We eat fish and many other things that are obtained from the aquatic environment. So, the lockdown condition also dramatically impacts the aquatic environment. Before the condition of this lockdown, people were straightforward and free to do their activities, and also, they were causing problems for the aquatic environment. Now, with a reduction in the activities like throwing the wastes in the aquatic sources, these sources are saved too much extent.

Impact on rivers

Rivers' pollution is different from the pollution in the ocean. Sea pollution is created by throwing billions of tonnes of trash into it, but river pollution is subject to industrialization and urbanization. Toxicity increases due to the poor filtration of the sewerage water mixed with the rivers such as the Ganga and Yamuna rivers; water quality has been significantly deteriorated due to these several toxic wastes from the systematic issues and immediate release of industrial waste into rivers (Arora *et al*., 2020).

"More than 38,000 million liters of untreated sewage are discharged daily into the rivers due to the limited sewage treatment capacity, which can treat only 38% of the sewage generated" (Balamurugan *et al.,* 2021). One study stated that around 10% of the toxic load in these rivers came from industries. 10% of this waste makes about 700 million liters of toxic water contamination into river water every day. Thus, creating the Ganga and Yamuna's water undrinkable and causing several chronic diseases, hepatitis, and multiple other diseases.

However, most of the waste in these historic rivers was from the sewerage water from the surrounding towns and villages. It makes more than 80% of the total pollution in these rivers. Moreover, due to 3 lockdowns stretching up to several months, the industrial waste in these rivers was massively reduced, and the water quality was improved in the last two years (Singhal and Matto, 2020). "The Ganga water at Haridwar and Rishikesh were found clean and safe for drinking due to 500% decrease in sewage and industrial effluents after lockdown due to COVID 19 pandemic" (Arora *et al.,* 2020).

Noise levels

Noise pollution is the third most dangerous pollutions after water and air pollution. During the lockdown, a significant decrease was measured in the noise levels. People are trapped in their homes due to government quarantine measures around the world. This global quarantine reduced the use of private and public transport and significantly reduced commercial activity. Due to the reduction in cars, motorcycles, buses, rickshaws, vans, airplanes, and overall decreased mobility also decreased the noise levels. Noise pollution from traffic is related to economic activity (Mishra *et al.,* 2021).

Therefore, the low demand for mobility was expected to significantly reduce traffic noise in the short term. This also reduced the effects of stress, anxiety, and depression in people. So, decreased noise levels have also improved the mental as well as the physical health of the people. When you hear a noisy sound, you become more restless and do the things that you should not do. Noise causes anxiety in a person. This is reduced with the help of lockdown. This is a significant factor that helped for the men. Health issues are reduced to some extent. This is very beneficial at the country level too. The funds of the country are left. This is the step for the country's development as well (Mishra *et al.,* 2021).

Wildlife:

The Wildlife worldwide seemed to regain their territory while the people around the world were trapped in their homes. Social media also showed the wild animals' pictures in the residential areas. The emergence of Wildlife in urban areas is primarily due to the tranquility that

attracts these animals to residential areas. Moreover, some animals, such as rats, monkeys, etc., that sometimes depend upon humans for food may suffer a little and try to find their food independently. A positive impact on Wildlife during lockdown was that they could breathe pollution-free air. This proved to be a practical step toward the health of Wildlife. In addition to this, the hunting of animals was also reduced due to the lockdown. This resulted in an increase in the population of Wildlife and betterment for the environment.

Soil health:

Soil elasticity is an important factor for overall soil quality or soil health. The most direct impact on the ground is caused by lockdown and decreased mobility. Human activity due to reduced human consumption leads to excess food. It is discarded and added to the soil. Decreased meat consumption is with food waste. This has heavily affected land use, groundwater quality and soil value. However, improvement in the underground water due to decreased water pollution has also affected to improve soil quality. When human activities are controlled to many extents, their wastes are less produced during the lockdown. This is proved to improve the fertility of the soil. The soil is the wealth of human beings. They get almost all the things from the soil.

This is a very beneficial thing which is called soil. The soil is the course of all the vegetables, fruits, and the major part of the food, the plants. Plants are of great importance in our daily life. It is proved very effective for the plants that the activities of the human beings are controlled. The activities of human beings are the major causing factor for the destruction of plants. As deforestation is the central part imposed the human beings to eradicate the plants and the trees for their benefit. In this way, the lockdown has proved very effective and beneficial for plants and soil fertility. Soil is wealth as we have saved our soil by any means like the lockdown. This will prove to be effective for Stratton. The increase in soil fertility will help us to the same extent in the fields of health and economy.

Forests:

Due to the lockdown, all the industries which reduced their manufactures caused little consumption of the forest wood. This step proved for the rehabilitation of the forest and also for the Wildlife. It also provided us with more clean air. With less consumption of trees, more oxygen was produced due to the forests, which provided the environment with more clean air to breathe. In addition to this, the betterment of the forests also proved to be very significant for the Wildlife to reside in. Hence, lockdown caused an improvement in the forests and the forest world. But on the other hand, the excess timber wood has caused draught (Adams, 2021).

As there is less consumption and need of lumber, so this is the slightly negative impact that leads to the draughts. Forests were damaged by the uncontrolled activities of human beings. This was the reason that the forests were being converted into deserts. So, the activities are e; also the forests are safe to much extent. But here is the point that when people live in their homes, the population level also increases. This increased population level is not suitable for plant life. The plants and trees will also be cut by human beings to make the space empty for their living mode. This is the second face of the picture that the lockdown Situation also has a negative impact too (Adams, 2021).

Impact on the virtual environment

Let me explain first that what the virtual environment is. The virtual environment is the internal environment of everything. You see in the world that there is an existing environment in which we live. This environment is seen able for us. But here is another environment that I mentioned the virtual environment. So, the lockdown has freshed the virtual environment as human beings are stared to live in the human beings. Also, the animals were afraid of the attacks of the humans every time.

They are happy now to some extent. They have not that fear. As we talk about plants, they do not flourish when human beings interrupt their growth and development. This is proved in the studies that the plants or the trees grow more when there are not interrupted by the activities of human beings. So, the virtual environment of the plants is also affected in an effective manner. Here is the positive impact of the lockdown condition on the virtual environment of the trees, plants, human beings, and many other creatures.

Air pollution

Lockdown during the global pandemic was the worst time for almost every economy of this world. However, it was a break for our mother planet earth and also provided a vital and rare opportunity for the earth to build a clean and pollution-free clear sky and air. Before the lockdown due to Covid-19, the whole world, specifically the South Asian region, including India, China, Bangladesh, and Pakistan, had the most polluted areas in the entire world, especially in the form of CO_2, NO_2, SO_2, and particulate matters. The primary reason behind the immense increase in the amount of these pollutants was the carbon emission from transport, industrial smoke, plants, and power stations. However, to control these pollutants in the past 2 decades, several agencies started emerging across the world that took the task of controlling the carbon emission and pollution levels.

Additionally, these programs started several programs responsible for the clean air levels that were not at the same quality of compared to the global organizations like WHO. Still, these programs and organizations were putting in significant effort to bring change to society. Either way, most of the companies were continuing their regular business conduct during 2001 and 2010. However, most of the global companies started investing in sustainable manufacturing and production methods to reduce carbon footprints. However, all of these efforts were just about the tip of the iceberg compared to the damage that has been done in the past several centuries. Damage can be portrayed in a way that a report of World Health Organization stated that approximately 4.2 million out of 60 million (annual death (8%) of total global deaths are due to air pollution (WHO, 2016).

Conclusion:

Lockdown had several terrible impacts on the overall world, including animals, aquatic life, and humans. However, the positive aspect of this pandemic was for the earth, which had been suffering from the issues like severe pollution, damaged ozone layer, global warming, and climate change. India has become one of the polluted countries in the world and has suffered a lot from the smog at the start of the winter season. However, the lockdown has proven to be a break for the earth to breathe release the toxic gasses caused by industries, transport, and carbon emission from a forest fire and other kinds of smoke. Moreover, still, humans continue to damage the water life by throwing millions of tonnes of additional plastic in terms of PPE kits. We've to stop these inhumane practices and find sustainable ways to biodegrade plastic to reduce the trash without creating an excessive carbon footprint in the air.

References:

Agnihotri, Nikhil, Dassani Sippy and Sharma T.K. (2020).tula[;k o`f) ,oa [kk| lqj{kk] dksfoM&19 ,oa 21oha lnh dk Hkkjr]Jigyasa XIV(1) pp. 95-100.

Agnihotri, Nikhil, Sharma T.K. and Sippy Dassani (2020). Population Growth, Food Security, Biodiversity and Covid-19; The Biobrio VII(3-4), pp. 503-508.

Agnihotri, Nikhil (2020). Corona Virus: A New Health Hazard, In Proceedings of National Conference on Recent Trends and New Frontiers in Biotechnology, Agricultural Science and Environment, Genome Biotech Publication, Mathura, ISBN: 978-81-92-9995-0-2, pp. 102-103.

Adams, M. A. (2021). Covid-19 pandemic impacts on forests. Frontiers. Retrieved January 3, 2022, from https://www.frontiersin.org/research-topics/14527/covid-19-pandemic-impacts-on-forests

Arora, S., Bhaukhandi, K. D., & Mishra, P. K. (2020, November 10). Coronavirus Lockdown helped the environment to Bounce Back. The Science of the total environment. Retrieved January 3, 2022, from https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7323667/

Balamurugan, M., Kasiviswanathan, K. S., Ilampooranan, I., & Soundharajan, B.-S. (2021). Covid-19 lockdown disruptions on Water Resources, wastewater, and agriculture in India. Frontiers in Water, 3. https://doi.org/10.3389/frwa.2021.603531

Edmond, C. (2020). How face masks, gloves and other coronavirus waste is polluting our ocean. World Economic Forum. Retrieved January 2, 2022, from https://www.weforum.org/agenda/2020/06/ppe-masks-gloves-coronavirus-ocean-pollution/

Explained Desk (2020, June 28). Covid-19 lockdown cleared up the air, but harmful ozone levels increased. here's why. The Indian Express. Retrieved January 3, 2022, from https://Indianexpress.com/article/explained/covid-19-lockdown-air-pollution-ozone-6479987/

Mishra, A., Das, S., Singh, D., & Maurya, A. K. (2021). Effect of covid-19 lockdown on noise pollution levels in an Indian city: A case study of Kanpur. Environmental Science and Pollution Research, 28(33), 46007–46019. https://doi.org/10.1007/s11356-021-13872-z

Singhal S., Matto M. Down to Earth; A blog. 2020. COVID-19 lockdown: a ventilator for rivers.

The world counts. (2022). About 60 million people die every year. That's about the number of people living in South Africa. Over half of all deaths worldwide can be attributed to 10 causes. Retrieved January 2, 2022, from https://www.theworldcounts.com/populations/world/deaths

WHO. (2016). Air pollution. Retrieved January 3, 2022, from https://www.who.int/health-topics/air-pollution#tab=tab_1

ANTIOXIDANT AND ANTIDIABETIC ACTIVITY OF FORMULATED PEARL MILLET ICECREAM

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Abstract:

Ice cream is considered a food for enjoyment, rather than a basic food. Enrichment with nutrients or other bioactive substances increases their nutritional quality. The study was aimed to formulate pearl millet ice cream and to study its functional properties as antioxidant and antidiabetic activity. The antioxidant activity was analysed using FRAP and DPPH assay and antidiabetic activity using α- Amylase inhibition and α- Glucosidase inhibition assay. Formulated pearl millet icecream possessed a high antioxidant activity (201 µg AAE in FRAP assay and 85.2 percentage inhibition in DPPH assay and showed a decreasing pattern in the antioxidant assay and antidiabetic activity on storage. The study revealed that formulated pearl millet milk ice cream possess a higher acceptability, nutritionally rich and functionally superior.

Keywords: Pearl millet, Icecream, Antioxidants, Antidiabetic

Introduction:

Driven by increasing incomes and health consciousness value addition to dairy products has witnessed a significant increase over the past few years. Icecream is a widely consumed food and is a recognized medium to help increase the milk intake and is a favourite dessert for men and women of all ages. Fortification of ice cream with nutrients or other bioactive substances should be supported (malik *et al.*, 2013).

Ice cream had its origins in Europe and was introduced later in the US where it was developed into industry. Ice cream plants were also established in Newyork, Saint Louis, Chicago, Washington and Cincinnati. Ice cream cone and Eskimo pie were introduced in 1904 and 1921, respectively (Goff and Hartel, 2013). In India ice cream is often served in weddings, in opening ceremonies and many other occasions. The ice cream business in India was approximately Rupees 2000 crore in the year 2013-2014. The per capita consumption of ice

cream in India is approximately 0.2 liter per annum while the average global per capita consumption is 2 liter.

Frozen desserts are valued by consumers for their wide range of excellent quality flavours. Addition of flavours also provides manufacturers with an opportunity to differentiate their products and compete for market share. The important flavouring agents are vanilla, chocolate and cocoa, confectionery and bakery inclusions, fruits and fruit extracts, nuts, spices although other flavours can be incorporated into frozen desserts. The ice cream industry is very progressive with many new product introductions annually. Some of the current formulation trends in the industry including a growing interest in "reduced" or "no" claims for fat, calories or sugar; the use of nutritionally functional additives (e.g. vitamins or minerals) or flavours showing added nutritional functionality (e.g. high in antioxidants) (Goff and Hartel, 2013).

Cereal based milk products are popular all over India. Cereals and milk are blended to compensate for deficiency of lysine (Aneja *et al.,* 2002). Some of the agricultural foods are not used as human main food because of unawareness of people. Millets are one of them. These are underutilized and neglected crop because of little knowledge to people and some critical problems like lower cooking quality and taste. These problems can be solved and make them valuable as food for poor families to combat malnutrition and important sources of income (Yang *et al.,* 2012).

Pearl millet (*Pennisetum glaucum*), also known as Bajra and Kambu, is one of the foremost important millets grown in tropical semi-arid regions of the world. Pearl millet is rich in several nutrients as well as non-nutrients such as phenols. It has high energy and fibre, has less starch, low glycemic index and is gluten free. The protein content ranges from 8 to 19% and the lipid content is about 3 to 6%. It can be used in a variety of ways including leavened and unleavened breads, porridges, biscuits, upma mixes, puffed snacks etc. It is also utilized as an ingredient in alcoholic beverages. Even though this millet is cost effective and abundantly available, it is underutilized and health facts of Bajra remain unknown. Owing to the benefits of pearl millet, its consumption should be encouraged among the people of all age groups to improve their dietary habits and nutritional status (Sindhu and Radhai, 2018).

With the increasing urbanization and the changing lifestyles, the consumption of coarse cereal has declined. To restore their lost importance, there is a need to expose these food grains in ready to eat forms and novelty products. The aim of this study was to increase the consumption of pearl millet by incorporating into ice-cream and to study the antioxidant activity and antidiabetic activity of the formulated pearl millet ice cream.

Methodology:

Pearl millet ice cream was prepared using the standard method substituting pearl millet milk instead of cow's milk. Take a small bowl and add milk & corn flour to it. Mix well and keep the mixture aside. Heat it over moderate flame and allow it to boil for 7-8 minutes, while stirring occasionally. Now add the sugar and cook on a medium flame for 5 minutes, while stirring continuously and cool completely. Once the mixture is cooled, add the fresh cream and flavours (pine apple pulp) using an electric beater blend it well. Pour the mixture into an aluminium container and cover with an aluminium foil. Freeze it for 6 hours or till set.

The pearl millet icecream was prepared in two variations –

Variation I (V l) with 100 % pearl millet milk

Variation II (V 2) with 50 % pearl millet milk + 50 % cow's milk

Functional Properties of Formulated Pearl Millet Ice Cream

The functional properties of the formulated pearl millet ice cream were analysed by its antioxidant activity and antidiabetic activity initially, 15 th day and on 30 th day .Pearl millets are having good antioxidant activity and phenolic content and thereby they can be used ingredients in functional food formulations.

Antioxidant activity of formulated pearl millet ice cream

Preparation of Sample

Allow the sample to soften at room temperature. It is not advisable to soften the icecream sampleby heating on water bath or over flame because melted fat tends to separate and rises to the surface. After softening of ice-cream, mix it thoroughly by stirring with spoon or egg beater or by pouring back and forth between beakers (IS, 1964).

Antioxidants are compounds that inhibit oxidation, especially one used to counteract the deterioration of stored food products. Pearl millet contains high levels of antioxidants namely the phenolic compounds especially flavonoids. The antioxidant activity of the formulated pearl millet icecream was determined using DPPH and FRAP assay

DPPH Radical scavenging activity

Different volumes (2 - 20µl) of sample extracts with ethanol were made up to 40µl with DMSO and 2.96 ml DPPH (0.1mM) solution was added. The reaction mixture was incubated in dark condition at room temperature for 20 min. After 20 min, the absorbance of the mixture was read at 517 nm by UV-Vis Spectrophotometer. 3ml of DPPH was taken as control.

$$\% \text{ RSA} = \frac{\text{Abs control} - \text{Abs sample}}{\text{Abs control}} \times 100$$

Where, RSA is the Radical Scavenging Activity; Abs control is the absorbance of DPPH radical + ethanol;Abs sample is the absorbance of DPPH radical + sample extract.

Ferric Reducing Antioxidant Power (FRAP) Assay

Ascorbic acid (vitamin C) was employed as a standard in this assay, and its calibration curve was obtained by using its concentrations ranging from 50 mg/L to 500 mg/L in water. To 2.85 mL FRAP reagent in a test tube, 150 µL sample (0.1 mg/mL, in methanol) or standard was added. The mixture was incubated for 30 min in the dark, and its absorbance was measured at 593 nm. The blank contained an equal volume of methanol instead of the sample. The results were reported as µg of ascorbic acid equivalents (AAE) per gm.

$$\text{FRAP Value (µg/g)} = \frac{\text{Abs sample x Frap value of Std(µg)}}{\text{Abs Std}}$$

Anti-diabetic activity of formulated pearl millet ice cream

Pearl millet is very powerful in controlling diabetes. Because of its high fibre content, it tends to digest slowly and release glucose at a lower level. It also contains low glycemic index 55 GI [13](Anonymous, 2016) and the antidiabetic activity was determined according to AOAC and expressed as percentage inhibition.

α-Amylase inhibition assay

Methanolic extract (100 µL, 1 mg/ml) was added with 100 µL of 0.02 M sodium phosphate buffer (pH 6.9) and 100 µL of α-amylase solution (4.5 Units/ml/min) and pre incubated at 25°C for 10 min. Then, 100 µL of 1% starch solution was added and incubated at 25°C for 30 min and the reaction was stopped by the addition of 1.0 ml of dinitrosalicylic acid reagent. The test tubes were then incubated in a boiling water bath for 5 min and then cooled to room temperature. The reaction mixture was diluted (10-fold) with distilled water and the absorbance was measured at 540 nm. The readings were related with the control, which contains buffer instead of extract and the percentage of α-amylase enzyme inhibition was calculated.

α- Glucosidase inhibition assay

Methanolic extract (100 µL, 1 mg/ml) was taken with 100 µl of 0.1 M phosphate buffer (pH 6.9) and 100 µL of α-glucosidase solution (1 Unit/ml/min) and pre incubated at 25°C for 5 min. Then, 100 µL of *p*-nitro phenyl-α-D-glucopyranoside (5 mM) was added and incubated at 25°C for 10 min. After the incubation period, the absorbance readings were recorded at 405 nm and allegorized to a control that had 100 µL of buffer in place of the extract. The results were calculated and expressed on percentage basis.

Results:

Antioxidant Activity of Formulated Pearl Millet Ice cream

Antioxidants are compounds that inhibit oxidation. Oxidation is a chemical reaction that can produce free radicals, thereby leading to chain reactions that may damage the cells of organisms

Antioxidant Activity of Formulated 100 % pearl millet ice cream (V1)

Antioxidant activity of 100 % pearl millet ice cream during the initial, fifteenth and thirtieth day is given in the table 1.

Table 1: Antioxidant Activity of Formulated 100% pearl millet ice cream (V1)

Antioxidant activity	Zeroth Day	Fifteenth Day	Thirtieth Day
DPPH (% inhibition)	85.2	82.0	79.9
FRAP (µg AAE)	201	186	176

Antioxidant activity of 100% pearl millet ice cream determined by DPPH and FRAP assay showed the highest values initially. DPPH method gives 85.2% inhibition and FRAP method gives 201µg AAE. On the fifteenth day it has been decreased to 82.0% inhibition (DPPH) and 186µg AAE (FRAP). On the thirtieth day the antioxidant activity of the ice cream again decreased to 79.9% inhibition (DPPH) and 176µg AAE (FRAP). A decreasing pattern in the antioxidant activity of the ice cream on storage was observed.

Antioxidant Activity of formulated 50% pearl millet ice cream (V2)

Antioxidant activity of 50% pearl millet ice cream (V2) during the initial, fifteenth and thirtieth day was given in the table 2.

Table 2: Antioxidant Activity of formulated 50% pearl millet ice cream (V2)

Antioxidant activity	Zeroth Day	Fifteenth Day	Thirtieth Day
DPPH (% inhibition)	80	76.0	72.0
FRAP (µg AAE)	133	124	120

Antioxidant activity of 50% pearl millet ice cream (V2) recorded highest as 80% inhibition on the initial day and FRAP method gives 133µg AAE. On the fifteenth day it has been decreased to 76.0% inhibition (DPPH) and 124µg AAE (FRAP). On the thirtieth day the antioxidant activity of the ice cream decreased to 72.0 % inhibition (DPPH) and 120µg AAE (FRAP).

Anti-diabetic Activity of formulated 100% pearl millet ice cream

Table 3 and table 4 presents the antidiabetic activity for the formulated 100% pearl millet ice cream and 50 % pearl millet ice cream.

Table 3: Anti diabetic Activity of formulated 100% pearl millet ice cream (V 1)

Anti-Diabetic Assay	Zeroth Day	Fifteenth Day	Thirtieth Day
α- Amylase inhibition (%)	78.6	76.0	73.8
α- Glucosidase inhibition (%)	81.5	78.2	77.0

The enzymes used in the assays are involved in the carbohydrate metabolism. Results found to be highest on initial day i.e. α- amylase inhibition gives 78.6% and α- glucosidase inhibition gives 81.5%. On the fifteenth it has been decreased to 76.0% (α- amylase inhibition) and 78.2% (α- glucosidase inhibition). On the thirtieth day it has been decreased to 73.8% α- amylase inhibition and 77.0% on α- glucosidase inhibition.

Table 4: Anti diabetic Activity of formulated 50% pearl millet ice cream

Anti-Diabetic Assay	Initial Day	Fifteenth Day	Thirtieth Day
α- Amylase inhibition (%)	73.5	69.5	66.8
α- Glucosidase inhibition (%)	77.2	71.0	70.1

The enzymatic assay showed an α- amylase inhibition of 73.5% and α- glucosidase inhibition gives 77.2%. It has been decreased to 69.5% and 71.0% respectively on fifteenth day and 66.8% and 70.1% on thirtieth day.

Conclusion:

Cereal based milk products are popular all over India. Dietary quality of food should be taken into consideration for maintaining over all maximization of human health and fitness to solve the problem of malnutrition. This study revealed that the formulated pearl millet ice cream were good sources of essential nutrients, organoleptically accepted, high antioxidant capacity and anti-diabetic agent and functionally superior.

References:

Aneja, R. P., Mathur, B. N., Chandan, R. C., and amp; Banerjee, A. K. (2002). Technology of Indian milk products: handbook on process technology modernization for professionals, entrepreneurs and scientists. Dairy India Yearbook

Goff H.D. and Hartel R.W (2013). Ice Cream, DOI 10.1007/978-1-4614-6096-1_1, Springer Science + Business Media New York

Indian standard specification for ice cream- IS 2802 (1964): Ice cream [FAD 19: Dairy products and equipment

Malik P, Kempanna C, Paul A (2013). Quality Characteristics of ice cream enriched with Spirulina powder. International journal of food and nutritional sciences. Vol 2, Issue 1, pp 44 – 50

Sindhu S. and amp; Radhai Sri S.(2018). Antioxidant Properties of Pearl Millet (*Pennisetum glaucum*) Volume-03, Issue-06, ISSN: 2455-3085 (Online), Research Review International Journal of Multidisciplinary

Yang X., Z. Wan, L. Perry, H. Lu, Q. Wang, C. Hao,J. Li, F. Xie, J. Yu, T. Wang, M. Li and Q.H. Ge.(2012) . Early millet use in northern china. Proc. Nat. Acad. Sci. USA pp. 1-5

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