PRACTICAL MANUAL

GROWTH AND DEVELOPMENT OF VEGETABLE CROPS

HVS-503 3(2+1)

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COURSE- GROWTH AND DEVELOPMENT OF VEGETABLE CROPS HVS-503 3(2+1)

Preparation of plant growth regulator's solutions and their application; Experiments in breaking and induction of dormancy by chemicals; Induction of parthenocarpy and fruit ripening; Application of plant growth substances for improving flower initiation, changing sex expression in cucurbits and checking flower and fruit drops and improving fruit set in solanaceous vegetables; Growth analysis techniques in vegetable crops; Grafting techniques in tomato, brinjal, cucumber and sweet pepper.

Name of Students .....

Roll No. .....

Batch .....

Session .....

Semester .....

Course Name : .....

Course No. : .....

Credit .....

CERTIFICATE

This is certify that Mr./Ms. ……ID No……has completed the practical of course **Growth and Development of Vegetable Crops, HVS-503** as per the syllabus of M.Sc. Vegetable Science, Semester……in the year……in the respective lab/field of college.

Date: Signature of Course Instructor

CONTENTS

Experiment No. 01

Objective: To prepare various standard solutions

Principle:

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Exercise 1. Prepare 0.1 N solution of NaOH

Material Required:

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Procedure: .....

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Calculations: .....

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Exercise 2. Prepare 0.2 M solution of NaCl

Material Required:

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Procedure: .....

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Calculations: .....

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Exercise 3. Prepare 10 ppm solution of $CuSO_4$

Material Required:

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Procedure: .....

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Calculations: .....

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Experiment No. 02

Objective: To prepare various hormonal solutions

Exercise 1. Prepare 100 ppm of NAA solution

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Procedure: .....

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Calculations: .....

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Exercise 2. Prepare 10 ppm of IBA solution

Material Required:

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Procedure: .....

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Calculations: .....

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Exercise 3. Prepare 0.05% solution of GA_3 hormone

Material Required:

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Procedure: .....

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Calculations: .....

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Experiment No. 03

Objective: Practical application of Plant growth regulators and growth retardants

(A) Auxin:.....

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Practical application.....

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(B) Gibberellins.....

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Practical application .....

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(C) Cytokinin:

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Practical application .....

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(D) Abscisic Acid:

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Practical application .....

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(E) Ethylene: .....

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Practical application .....

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Practical application of growth retardants.....

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Experiment No. 04

Objective: To extract and estimate the chlorophyll content in given plant tissues

Material Required:

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Procedure: .....

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Formula for calculation: The spectrophotometer is calibrated by using 80% acetone as blank sample.

$$\text{Chlorophyll a} = 12.7\,(A663) - 2.69\,(A\,645) \times \frac{V}{1000 \times W}$$

$$\text{Chlorophyll b} = 22.9\,(A\,645) - 4.69\,(A\,663) \times \frac{V}{1000 \times W}$$

$$\text{Total chlorophyll} = \frac{A652 \times 1000}{34.5} \times \frac{V}{1000 \times W}$$

Where A= Optical density

V= Final volume of leaf sample (25 ml)

W= Weight of leaf tissue (in gm i.e. 025 g)

The chlorophyll content of the leaf sample is expressed as **mg**/ **g** of fresh leaf

Calculation:

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Experiment No. 05

Objective: To extract and estimate the carotenoids content in given plant tissues

Material Required:

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Procedure: .....

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Formula for calculation: The spectrophotometer is calibrated by using 80% acetone as blank sample.

$$\text{Carotenoids:} \quad \frac{7.6\,(A\,480) - 1.49\,(A\,510)\,V}{1000 \times W}$$

Where A= Optical density

V= Final volume of leaf sample

W= Weight of leaf tissue (in gm)

The carotenoid content of the leaf sample is expressed as **mg**/ **g** of fresh leaf

Calculation:

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Experiment No. 06

Objective: Measurement of photosynthesis efficiency in variable environments

Material Required:

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Procedure:.....

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Observation:

Plant Species	Fv	Fv	Fv/FM

Conclusion.....

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Experiment No. 07

Objective: Methods of breaking seed dormancy

Methods for breaking Hard Seededness

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Methods for breaking Physical dormancy

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Observation:

Seeds	Treatments	Duration	Germination percentage

Experiment No. 08

Objective: To determine the seed viability by tetrazolium test

Material Required:.....

Reagents::.....

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Procedure: .....

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Observation:.....

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Seed sample	Total No. of Seeds tested	No. of Viable Seeds	No. of Non-Viable Seeds

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Experiment No. 09

Objective: To determine the seed viability by gemination test

Material Required:

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Procedure: .....

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Observation:.....

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Seed sample	Total No. of Seeds tested	No. of Seeds germinated	Germination %

Calculation: Germination % = [Germinated seeds/ Total number of seeds] x 100

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Experiment No. 10

Objective: Induction of parthenocarpy and fruit ripening in vegetable crops

(a) Induction of parthenocarpy

Principle:

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Material Required:……..
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Procedure:

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Conclusion:

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(b) Induction of fruit repining

Principle:

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Material Required:……..
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Procedure:

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Conclusion:

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Experiment No. 11

Objective: Application of plant growth substances for improving flower initiation, changing sex expression in cucurbits

Principle:

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S. No.	Crops	PGR's	Effect

Experiment No. 12

Objective: Application of plant growth substances to check flower and fruit drops and improving fruit set in solanaceous vegetables

Principle:

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Application of Plant growth substances

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S. No.	Crops	PGR's	Effect on fruit drops and fruit set

Experiment No. 13

Objective: To measure the leaf area by various method

1. Graphic method

Material Required:

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Procedure: .....

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Calculations: Count the squares of estimated leaves and calculate the leaf area in cm^2.

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2. Dry weight method

Material Required:

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Procedure: .....

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Calculations:

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3. Linear method

Material Required:

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Procedure: .....

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Calculations:

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Experiment No. 14

Objective: To study the growth analysis parameters; CGR, RGR, AGR, NAR

Exercise 1: Plant dry weights of bitter gourd at 15, 30 and 45 DAS are given in the following table. Calculate the CGR, if spacing is 30 cm x 10 cm

Plant dry weight (g/plant)		
15 DAS	30 DAS	45 DAS
0.27	2.16	4.71

Calculations:

CGR between15-30 DAS:

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CGR between 30-45 DAS: .....

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Exercise 2: Plant dry weights of bitter gourd at 15, 30 and 45 DAS are given in the following table. Calculate the RGR.

Plant dry weight (g/plant)		
15 DAS	30 DAS	45 DAS
0.27	2.16	4.71

Calculations:

RGR between 15-30 DAS:

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RGR between 30-45 DAS: .....

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Exercise 3 Plant dry weights of bitter gourd at 15, 30 and 45 DAS are given in the following table. Calculate the AGR.

Plant dry weight (g/plant)		
15 DAS	30 DAS	45 DAS
0.27	2.16	4.71

Calculations:

AGR between 15-30 DAS:

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AGR between 30-45 DAS: .....

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Exercise 4: Leaf area and leaf dry weight of bitter gourd at 15, 30 and 45 DAS are given in the following table. Calculate the NAR.

15 DAS		30 DAS		45 DAS	
Leaf area (cm^2/plant)	leaf dry weight (g/plant)	Leaf area (cm^2/plant)	Leaf dry weight (g/plant)	Leaf area (cm^2/plant)	Leaf dry weight (g/plant)
45.37	0.16	361.84	1.22	475.82	1.71

Calculations:

NAR between 15-30 DAS: .....

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NAR between 30-45 DAS: .....

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Experiment No. 15

Objective: To study the growth analysis parameters; LAI, LAR, LAD, SLA, SLW, Harvest index

Exercise 1: Leaf area of bitter gourd at 15, 30 and 45 DAS are given in the following table. Calculate LAI, if the spacing is 30 cm x 10 cm.

Leaf area (cm^2/plant)		
15 DAS	30 DAS	45 DAS
45.37	361.84	475.82

Calculations:

LAI at 15 DAS

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LAI at 30 DAS

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LAI at 45 DAS

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Exercise 2: Plant dry weight and leaf area of bitter gourd at 15, 30 and 45 DAS are given in the following table. Calculate the LAR.

15 DAS		30 DAS		45 DAS	
Leaf area (cm^2/plant)	Plant dry weight (g/plant)	Leaf area (cm^2/plant)	Plant dry weight (g/plant)	Leaf area (cm^2/plant)	Plant dry weight (g/plant)
47.37	0.27	361.84	2.16	475.82	4.71

Calculations:

LAR at 15 DAS..... .....

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LAR at 30 DAS .....

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LAR at 45 DAS .....

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Exercise 3: Leaf area index of bitter gourd at 15, 30 and 45 DAS are given in the following table. Calculate the LAD.

Leaf Area Index		
15 DAS	30 DAS	45 DAS
0.15	1.21	1.59

Calculations:

LAD between 15-30DAS: .....

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LAD between 30-45 DAS: .....

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Exercise 4: Leaf dry weight and leaf area of bitter gourd at 15, 30 and 45 DAS are given in the following table. Calculate the SLA.

15 DAS		30 DAS		45 DAS	
Leaf area (cm^2/plant)	Leaf dry weight (g/plant)	Leaf area (cm^2/plant)	Leaf dry weight (g/plant)	Leaf area (cm^2/plant)	Leaf dry weight (g/plant)
45.37	0.16	361.84	1.22	475.82	1.71

Calculations:

SLA at 15 DAS:.....

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SLA at 30 DAS:.....

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SLA at 45 DAS:.....

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Exercise 5: Leaf dry weight and leaf area of bitter gourd at 15, 30 and 45 DAS are given in the following table. Calculate the SLW.

15 DAS		30 DAS		45 DAS	
Leaf area (cm^2/plant)	Leaf dry weight (g/plant)	Leaf area (cm^2/plant)	Leaf dry weight (g/plant)	Leaf area (cm^2/plant)	Leaf dry weight (g/plant)
45.37	0.16	361.84	1.22	475.82	1.71

Calculations:

SLW at 15 DAS:.....

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SLW at 30 DAS:.....

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SLW at 45 DAS:.....

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Exercise 6: Calculate the harvest index of the wheat if grain yield is 4000 kg/ha and biomass is 9000 kg/ha.

Calculations:

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Experiment No. 16

Objective: Grafting techniques in tomato and brinjal

Introduction:

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Pre-requisites for vegetable grafting:

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Grafting Methods:

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(f).....

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Grafting methods and rootstocks used in vegetable crops:

Scion Plant	Rootstocks	Methods

Experiment No. 17

Objective: Grafting techniques in cucumber and sweet pepper

Introduction:

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Pre-requisites for vegetable grafting:

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Grafting Methods:

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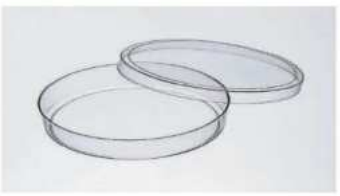
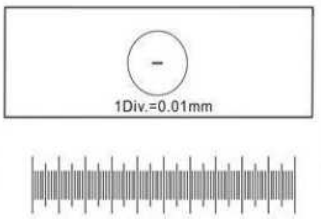
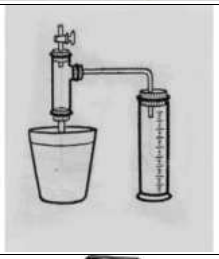
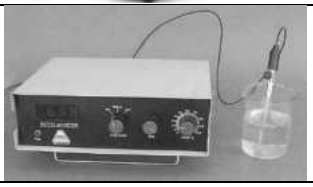
Grafting methods and rootstocks used in vegetable crops:

Scion Plant	Rootstocks	Methods

APPENDICES

IDENTIFICATION OF EQUIPMENTS/GLASSWARES

	Equipment /glassware	
1.	**Petri-plate/Petri-dish** It is small shallow dish of thin glass or plastic with a loose cover used especially for cultures in bacteriology. It is also commonly used as temporary receptacles for viewing samples, especially plant tissue or specimen under microscope.	
2.	**Pestle & Mortar** The mortar is a like bowl, typically made of hard wood, metal, ceramic, or hard stone, such as granite. The pestle is a heavy and blunt club-shaped object. The substance to be ground, which may be wet or dry, is placed in the mortar, where the pestle is pressed and rotated onto it until the desired texture is achieved	
3.	**Measuring Cylinder** A graduated cylinder, measuring cylinder or mixing cylinder is a common piece of laboratory equipment used to measure the volume of a liquid. It has a narrow cylindrical shape. Each marked line on the graduated cylinder represents the amount of liquid that has been measured.	
4.	**Spatula** Spatula is a broad spoon like end at one side and flat end at another side made of steel, it is used to lift or take chemical materials for the laboratory purpose	
5.	**Scalpel** A scalpel, is a small and extremely sharp bladed instrument like knife used for anatomical dissection or cutting of tissue/specimens. Scalpels may be single-use disposable or re-usable	
6.	**Cork Borer** Cork borer is a metal tool for cutting a hole in a plant tissue material like potato or any other materials for experiment purpose. It is usually come in a set of nested sizes along with a solid pin for pushing the removed cork (or rubber) out of the borer. The individual borer is a hollow tube, tapered at the edge, generally with some kind of handle at the other end.	
7.	**Stage Micrometer** A Stage Micrometer is simply a microscope slide with a finely divided scale marked on the surface. The scale is of a known true length and is used for the calibration of optical systems with eyepiece graticule patterns. It is a glass slide on which a 1 mm scale is accurately ruled into 10 equal divisions of 1/10 mm. Each division is subdivided into 10, so that each small division equals 1/100 mm (10 µm).	
8.	**Centrifuge** Centrifuge is a laboratory equipment, driven by a motor, which spins liquid samples at high speed. There are various types of centrifuges, depending on the size and the sample capacity. Laboratory centrifuges work by the sedimentation principle, where the centrifugal acceleration is used to separate substances of greater and lesser density	

9.	**Hot Air Oven** Hot air ovens are electrical devices which use dry heat to sterilize. Generally, they can be operated from 50 to 300 °C, using a thermostat to control the temperature.	
10.	**Ganong's Potometer** It consists of a graduated tube dipped into the beaker containing water. The graduated tube is connected with a vertical arm bearing a cork on its mouth. The cork contains one hole through which a twig is inserted in the water of the vertical arm. Vertical arm is also attached with a stop cork connected with a water reservoir. It is used for measure the rate of transpiration	
11.	**Ganong's Respirometer** Respirometer is an apparatus used to measure the rate of respiration of a living substance/tissue by measuring its rate of exchange of oxygen and/or carbon dioxide.	
12.	**Root Pressure Manometer** Manometers are used to measure the pressures at which water is forced into the xylem. If a root pressure manometer is attached to the cut stem, the root pressure can be measured. Root pressure is caused by active distribution of mineral nutrient ions into the root xylem.	
13.	**Water Bath** A water bath is laboratory equipment made from a container filled with heated water. It is used to incubate samples in water at a constant temperature at defined time. It is also used to enable certain chemical reactions to occur at high temperature.	
14.	**Compound light Microscope** A compound microscope is an upright microscope that uses two sets of lenses (a compound lens system) to obtain higher magnification than a stereo microscope. Compound microscopes typically provide magnification in the range of 40x-1000x. Compound microscopes are used to view small samples that cannot be identified with the naked eye. These samples are typically placed on a slide under the microscope	
15.	**pH Meter** pH meter is electric device used to measure hydrogen-ion activity (acidity or alkalinity) in solution. Fundamentally, a pH meter consists of a voltmeter attached to a pH-responsive electrode and a reference (unvarying) electrode.	
16.	**Infra-red Gas Analyser (IRGA)** A Photosynthesis system is design for the non-destructive measurement of photosynthetic rates in the field. Type of analysis possible- • CO_2 assimilation rates, • Stomatal conductance, • Carboxylation and light use efficiencies • CO_2 and light compensation points • PAR (photosynthetically active radiation)	

PREPARATION OF STANDARD SOLUTIONS

A standard solution contains a known weight of the substance is known volume of solvent.

1. **Percent (%) solution:** It contains in 100 ml of solvent, particular grams of a solute which is denoted by the percentage. The percentage of a solution can be expressed in three way:

   Weight per unit weight (w/w)- A 1% w/w has 1 g of solute and 99 g of solvent to make 100g of solution.
   Weight per unit volume (w/v)- A 1% of w/v solution contains 1 g of solute in 100 ml of solution.
   Volume by volume (v/v)- A 1% of v/v solution contains 1 ml of solute in 100 ml of solution.

   E.g., for preparing 10% NaCl, 10 g of the salt is dissolved in water and the volume is made to 100 ml. 10% Glycerol will contain 10 ml Glycerol and 90 ml water.

2. **Parts per million (ppm) solution:** 1 ppm solution is prepared by dissolving 1 mg of solute to 1 litre of solvent. For solutions 1 ml is diluted to 1 litre.

3. **Normal solution:** Normal solution contains in 1 litre of solution, one-gram equivalent of dissolved substance. One-gram equivalent of a substance corresponds to its molecular weight expressed in grams divided by its velency, e.g., 1 N Na OH contains 40 g NaOH in 1 litre. 1 N HCl may be prepared as follows:

   36.5 g of HCl per litr5e make a normal solution. Converting weight into volume = 36.5/1.16 = 31.4 ml. (Sp. Gravity of HCL = 1.16). The purity of HCl is 26 to 28%, hence (100/28) x 31.4 = 112.1 ml. Approximately 113 ml HCl dissolved per litre will give strength equal to 1 N.

4. **Molar solution:** A molar solution of a compound is defined as one mole of that compound per litre. 1 mole/1=molecular weight in grams/litre of solution, e.g., a solution of 0.1 M H_2SO_4, molecular weight 98 contains 9.80 g H_2SO_4, in 1 litre of solution. When 95% H_2SO_4, is available, the required 0.1 M is prepared as follows:

   95 g H_2SO_4, is contained in 100 g 95% H_2SO_4,
   9.8 g H_2SO_4, is contained in x g 95% H_2SO_4,
   Then x = (9.8 x 100)/ 95 = 10.315 g.
   Converting weight into volume = 10.315/1.84 specific gravity of H_2SO_4, = 5.605
   Thus 5.605 ml of 95% H_2SO_4, is diluted to 1 litre with water to obtain 0.1 M H_2SO_4,

Dilution of stock solutions: The stock solution of 1000 ppm can be diluted further to the required concentrations.

Suppose the required concentration is 25 ppm.
Stock concentration/ Required concentration =1000/25 = 40
Therefore, to dilute 1000 ppm stock to 25 ppm solution, dilute 1 ml of the stock solution to 40 ml with water.

PREPARATION OF HORMONAL SOLUTIONS

The strength of growth regulators is calculated in ppm (parts per million). One ppm means 1.0mg of chemical dissolved in one litre of water. After weighing the required quantity of growth regulator transfer it to a beaker and dissolve it with the small quantity of solvent.

- Auxins are soluble in alcohol or 0.1% NaOH.
- Gibberellins are soluble in absolute alcohol, while, cytokinins can be dissolved in 1-2 ml N/10 HCl.
- Abscisic acid is highly soluble in NaOH. Shake the beaker till the growth regulator/chemical is fully dissolved.
- Now transfer it into volumetric flask and make final volume with distilled water to one litre.

ESTIMATION OF CHLOROPHYLL PIGMENT IN PLANT TISSUES

Chlorophyll pigment is a large molecule with a tetra pyrrol ring and a magnesium ion held in it. Attached to one of the rings is a long insoluble hydrocarbon ring, a 20-carbon phytol
group. Chlorophyll b has a -CHO group in the third carbon of second pyrrol ring instead of -CH3 group as in the case of chlorophyll a.
Chlorophyll a and chlorophyll b have typical absorption spectra of solar radiation. Maximum peak of chlorophyll a is observed in blue violet (429 nm) and in red region (660 nm) while the chlorophyll b absorbs at 453 nm and 642 nm. Chlorophyll a is usually blue green and chlorophyll b is yellow green in colour. The formula for the chlorophyll a molecule is $C_{55}H_{72}O_5N_4Mg$ and chlorophyll b molecule is $C_{55}H_{70}O_6N_4Mg$.
MATERIALS REQUIRED: Fresh leaf, 80 % acetone, distilled water, balance, spectrophotometer, mortar and pestle
PROCEDURE:

- Take 250 mg of leaf sample and macerated with 10ml of 80% acetone using a pestle and mortar
- Extract or slurry is centrifuged at 3000 rpm for 10 minutes.
- The supernatant solution is transferred into a 25ml test tube and made up to 20ml using 80% acetone.
- The color intensity of the green pigment is read at 645nm, 663nm and 652nm for chlorophyll a, chlorophyll b and total chlorophyll content respectively using spectrophotometer against the solvent (80% acetone) blank.

ESTIMATION OF CAROTENOIDS

The Carotenoids are a group of yellow, orange and orange red fat-soluble pigment widely distributes in nature. Carotenes are hydrocarbons with the empirical formula C40H56 composed of eight isoprene units. Most xanthophylls have the formula $C_{40}H_{56}O_2$ and are yellow to brown in colour. Xanthophylls can be separated physically from carotene because they are more soluble in alcohol and much less soluble in petroleum ether.

MATERIALS REQUIRED: Fresh leaf, 80% acetone, distilled water, balance, spectrophotometer, mortar and pestle, Centrifuge

PROCEDURE:

1. Take 150 mg of leaf sample and macerated with 10ml of 80% acetone using a pestle and mortar
2. Extract or slurry is centrifuged at 3000 rpm for 10 minutes and the residue is reextracted with another 5ml of 80% acetone until homogenate becomes colorless
3. The supernatant solution is transferred into a test tube and make up volume to 15ml using 80% acetone.
4. The optical density of the extract is measured at 480 and 510nm wavelength in a Spectrophotometer against the solvent (80% acetone) blank.

PRACTICAL APPLICATION OF PLANT HORMONES AND GROWTH RETARDANTS

Plant growth regulators have been an important component in agricultural production even prior to the identification of plant hormone. Plant growth regulators are now used on over one million hectares worldwide on a diversity of crops each year. However, most of these applications are confined to high-value horticultural crops rather than field crops, although there are several significant exceptions.

Practical use of plant growth regulators:

Auxin: The auxin-type plant growth regulators comprise some of the oldest compounds used in agriculture. A number of synthetic compounds were found to act similarly to IAA in the auxin bioassay tests. Indolebutyric acid (IBA) and NAA were found to increase root development in the propagation of stem cuttings. 2,4-dichlorophenoxyacetic acid (2,4-D) stimulates excessive, uncontrolled growth in broadleaf plants for which it is used as a herbicide. NAA is used to reduce the number of fruits that have set in apple, whereas 4-chlorophenoxyacetic acid (4-CPA) is used to increase fruit set in tomato. The auxins 2,4,5-trichlorophenoxypropionic acid (2,4,5-TP) and the dichlrophenoxy analog (2,4-DP) are used to prevent abscission of mature fruit in apple.

Gibberellins: There are about 120 gibberellins found in both higher plants and the Gibberella fungus, although only two commercial products are available, GA3 and a mixture of GA4 and GA7. Both are produced by fermentation cultures of the fungus. GA is used extensively on seedless grape varieties to increase the size and quality of the fruit. some citrus species, can be induced to set fruit with GA, or a combination of GA and auxin. GAs is used to delay fruit ripening in lemon in order to increase the availability of fruit. GA application is used to increasing sugarcane yield. GA is used to increase the yield of barley malt. GA has also been used to control flower sex expression in cucumbers and squash. GA application tends to promote maleness in these plants.

Cytokinins: Benzyladenine is used on white pine to increase lateral bud formation and subsequent growth and branching. Pomina, a mixture of benzyladenine and GA4/7 is used to control fruit shape in 'Delicious' apple. Pomina is also being used to increase lateral branching in non-bearing apple trees. Cytokinins can also delay the senescence of cut flowers and fresh vegetables. Cytokinin is quite effective in breaking seed dormancy and some other plant organs. It also enhances femaleness in some plants like cucumber.

Abscisic acid: ABA induces seed dormancy. It maintains dormancy in potato tubers and buds. ABA is regarded as stress hormone, when a plant deficient in water, the ABA content of leaves rises rapidly. This action closes the stomata rapidly. Its can enhance ethylene formation and stimulates abscission. ABA induces flowering in SD plants and inhibits the same in long day plants.

Ethylene: Application of ethylene hastens ripening of climacteric fruits such as banana, mango, apple, tomato etc. It causes flowering in pine apple and shift the sex ratio of flowers towards femaleness in several cucurbits and cannabis. Application of ethephon helps in thinning of fruits in apple eliminates biennial bearing and also improve fruit size and quality. Use of ethylene increases latex flow in Havea and rubber yield by 50-80 %.

ESTIMATION OF LEAF AREA OF PLANTS BY VARIOUS METHOD

Leaf is assigned as one of the important organs of plant system and further development of plant depends upon the persistence of leaves. Physiologically, leaf area constitutes the main photosynthetic surface and supplies most of the photosynthates required by the seed, fruit or any storage organs. So, the estimation of leaf area is an essential integral part of classical growth analysis and is often important in physiological reasoning of variations in crop productivity. For the estimation of leaf area, several methods have been developed.

1. Graphic method
2. Dry weight method
3. Linear method
4. Leaf Area Meter

1. Graphic method: This involves the use of graph papers for the estimation of only smaller leaves or irregular shaped leaves (e.g., Castor, papaya etc.). So, this type of method cannot be used for estimating the leaf area for all types of leaves. For estimating the leaf area, the outline of the leaf is drawn on a graph paper and the number of full squares, half squares and quarter squares are counted and added. The leaf area is expressed as cm^2 per leaf.

Requirements: Fresh leaves of various species, graph paper, scale, pencil

Procedure: Place the leaf whose area is to be measured on graph paper and draw its outline. Remove the leaf and mark the squares (cm) within the outline of the leaf as:

Complete square (A) = 1 cm^2

Half the square (B) = 0.5 cm^2

More than half the square (C) = 1 cm^2
Less than half the square (D)=zero cm^2
Calculate the area of the leaf as: A + B + C + D = X cm^2

2. Dry weight method: The leaf area (L1) occupied by known dry weight (W1) of the single leaf can be found out adopting any one of the methods. This forms the basis for calculating the whole plant leaf area (L2) based on the total dry weight (W2) by using the given formula.

Total leaf area (L2) = $\frac{L1}{W1}$ x W2

Where, L2 - Total leaf area; W1 - Single leaf weight (dry)
L1- Single leaf area, W2 - Total leaf weight (dry)

3. Linear method: This method is relatively simple, time saving and non-destructive method for estimating the leaf area of regular shaped leaves (e.g., mango, wheat etc.). Montgomery (1911) studied the statistically defined mathematical relationship between the linear dimensions of the leaf area and proposed the following formula.

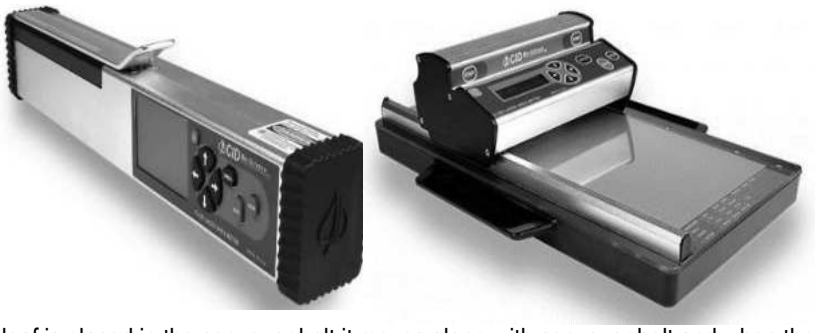
Leaf Area (A) = K x L x B

Where,

A = leaf area per leaf
L = maximum length of the leaf
B = maximum breadth of the leaf
K = leaf area constant (**0.75** is a constant evolved after experimentation).

The value of leaf area constant (K) is the ratio between actual leaf area and apparent leaf area and is always less than 1.

4. Leaf Area Meter (Electronic Method)



Leaf Area Meter is used for estimating leaf area of all types of leaves. This method is also termed as direct method. But the leaves should be removed or detached from the plants and fed into to the area meter. The estimation can be done only in the laboratory. In the area meter, fluorescent light source, mirror and scanning camera and a conveyor belt are provided. Initially zero is set. When a leaf is placed in the conveyor belt it moves along with conveyor belt and when the leaf comes close to scanning camera, it reflects the image of leaf on the mirror and the reading is measured digitally. Area of leaf is expressed in cm^2.

SEED VIABILITY BY TETRAZOLIUM TEST

A viable seed is one which is capable of germinating under the favourable conditions. Such a viable seed may or may not be readily or immediately germinable. Tetrazolium test is very useful for rapidly obtaining an indication of germination potential and viability of samples. A colourless solution of 2,3,5-triphenytetrazolium chloride reacts with hydrogen released by the reduction process in living cells due to the action of enzyme dehydrogenase. It produces a red and non-diffusible substance triphenylformazone to distinguish stained living cells from the colourless dead ones.

MATERIALS REQUIRED: Seeds, beaker, distilled water, petri dish, forceps

REAGENTS: 1. Phosphate buffer solution (pH 7.0). (A) Dissolve 9.087 g of KH2PO4 in 1 liter of water. (B). Dissolve 11.876 g of Na2HPO4.2H2o in 1 liter of water. Take 400 ml of solution A and 600 ml of solution B and mix them together.

2.Tetrazolium Solution (1%): Dissolve 10 g of TZ salt in a liter of buffer solution.

PROCEDURE:

1. Take seed and soak them in water overnight at room temperature.
2. Next day cut the seeds half to expose the embryo (in case of monocot), In case of dicot, remove the seed coat to facilitate the quick penetration of tetrazolium.
3. Keep the seeds in Petri plate and soak in 1% tetrazolium solution and keep in dark for 3-4 hours at room temperature for colour development.
4. When the colour is developed, drain out TZ solution, rinse seeds 2-3 times with water and evaluate the staining pattern. During evaluation put the seed in water.

Observation: Red stained area indicates the living tissues, while unstained area represents dead tissues. If entire embryo stains bright red in colour, it indicates that seeds are viable. Group the seeds in three categories 1. Completely stained seeds 2. Completely unstained seeds 3. Partially stained seeds. And calculate the percent seed viability.

SEED VIABILITY BY GERMINATION TEST

MATERIALS REQUIRED: Seeds, beaker, distilled water, petri dish, filter paper

PROCEDURE:

1. Take filter paper and cut according to the size of petri dish and place a double layer of filter paper in each dish.
2. Label each petri dish with sample number, replication and date and moist the paper with water.
3. Take 40 seeds randomly for the test and divide into at least 2 replications.
4. Arrange the seeds in a regular equidistant pattern on the surface of the paper.
5. Cover it and keep in the incubator or at room temperature for the definite time depending upon the crop.

Observation: After incubation period, count the number of fully formed seedlings. Normal seedlings are those which have well developed root system, shoot system and healthy.

BREAKING OF SEED DORMANCY

Inability of a viable seed to respond to the favourable environmental conditions for germinations is known as seed dormancy. In this condition seed is not able to germinate. Dormancy is generally of three types-hard seededness, physiological dormancy and presence of inhibitory substances.

Methods for breaking hard-seededness

Seed soaking: Dormant seeds are soaked in distilled water for 24-48 hrs and germination test is performed just after soaking.

Mechanical Scarification: Seeds of some crops scarified mechanically to break the hard seededness by the following method:

a). Piercing: Seed coat is pierced with the help of a sharp needle or knife without any injury to other part of seeds

b). Chipping: Seed coat is chipped with the help of sharp razor without injury to other part of the seeds.

c). Filling: Seed coat is scratched at the suitable site with a file or sand paper for entry of water.

Acid scarification: Seeds are soaked for a prescribed period in concentrated sulphuric or nitric acid to make the seed coat permeable to water and gases. After treatment seeds are thoroughly washed under running tap water for several times and tested for germination.

Methods for breaking physiological dormancy

1. **Dry storage:** Seeds of the crops having dormancy for short period of time are safely stored up to that period to break the dormancy e.g., sunflower
2. **Stratification:** Exposing imbibed seeds to low temperature (3-7°C) for prescribed time prior to germination in order to break the dormancy is known as stratification.
3. **Potassium nitrate:** A solution of potassium nitrate (0.2 %) is prepared by dissolving 2 g KNO3 in one liter of water. The seeds are treated with this solution and then tested for germination e.g., Brassica, tomato, and chilli
4. **Gibberellic acid:** GA_3 solution of 0.05% is prepared by dissolving 500 mg GA_3 in one liter of water. Seeds are treated with this solution and placed in the germinator e.g., wheat, barley, oat.

HORMONAL INFLUENCE ON CONTROL OF FLOWER AND FRUIT DROPS

Many fruit tree species bear an abundance of flowers which, even after poor pollination conditions, produce a surplus of fruit that the tree is unable to support. Possibly in anticipation of this, many fruit trees have developed a self-regulatory-mechanism whereby they shed part of their fruit load at a certain early period. From a horticultural point of view, this self-regulatory mechanism may be too strong for fruit species, such as mango, avocado etc., leading to low fruit load and yield. To overcome these shortcomings, flower or fruit thinning is an efficient method and has become necessary in modern fruit production. However, manual thinning is becoming more and more uneconomical, leaving thinning with bioregulators (PGR as well as endogenous plant hormones) as the only presently available alternative.

Chemicals used for thinning:

Napthaleneacetimide (NAD)

- Napthaleneacetimide is a less potent form of NAA.
- It frequently is used in situations where foliar damage caused by NAA is a problem, especially for summer cultivars.
- NAD is applied at 25 to 50 ppm at petal fall, or in a post-bloom spray when the fruit lets are 10-12mm in diameter.

Benzyladenine (BA)

- BA is marketed under the trade name Accel
- While Accel is not a strong thinner, it can promote increased fruit size and return bloom.
- To ensure adequate thinning, try the following sequence of thinning sprays.
- At petal fall, apply carbaryl.
- When fruit are between 5 and 15mm diameter, apply Accel at 30 grams active ingredient (a.i.) per acre.
- If fruit set appears heavy, include carbaryl in this second thinning spray.

Naphthaleneacetic acid (NAA)

- Naphthaleneacetic acid (NAA) is a powerful fruit thinning agent.
- NAA should be applied at concentrations of 2.5 to 20 ppm, depending upon the cultivar to be thinned and whether or not it is used in combination with carbaryl.
- When the fruitlets are 10-12mm in diameter, which usually occurs by 14 to 21 days after full bloom.

Summary of growth regulator treatments used for flower and fruit drops

Growth Regulator	Concentration	Timing	Effect
GA	1-2 sprays of 25 ppm at two weeks interval	Early June and at bud break	Reduce flower number to increase fruit size
GA	10-25 ppm	70-90 % petal fall	Improve fruit set
Ethephon	50-75 ml/L	When fruits are 10-15 mm diameter	Thin crop load and prevent fruit drop
3,5,6-TPA	15 ppm	When fruits are 15-20mm diameter	Thin crop load especially smaller fruitlets
3,5,6-TPA	15 ppm	When fruits are 20-30mm in diameter	Increase fruit size
2,4-D	55-110 g/ha @ 5000 L/ha	When fruitlets are 5-20mm in diameter	Increase fruit size
NAA	200-350 ppm	When fruitlets are 20mm in diameter	Fruit thinning

PLANT GROWTH ANALYSIS

Growth is a characteristic of life. It is the foremost symbolization of life in action. Growth continues till the end of an organism. It is expressed as height, weight (size), volume, number and area. Growth is always phenomenal. If growth stops vertically it may commence horizontally. If leaf expansion stops, it starts gaining in number and volume. Growth rate is quantified mathematically in terms of time as in cases of other vital processes of plants and this gives us a valuable information documenting growth as influenced by various abiotic, biotic, edaphic and seasonal factors. Growth analysis can be made at individual plant level or of plant communities.

Analysis of Individual plant growth, generally made at the early stage includes relative and absolute growth rate, net assimilation rate, leaf area ratio, specific leaf area, specific leaf weight and allometry (shoot/root ratio). Parameters used in growth analysis of plant communities includes leaf area index, leaf area duration and crop growth rate.

The technique of growth analysis is advantageous to plant scientists as it helps

1. To find out the relationship between photosynthetic production and rate of increase in dry matter.
2. To investigate the ecological phenomenon and competition between different species.

3. To predict the effect of agronomic manipulation.

Parameters of Growth Analysis

1. **Leaf Area Index (LAI):** Leaf area index (LAI) expresses the ratio of leaf surface to the ground area occupied by the plant. LAI is the measure of available photosynthetic surface per unit land area.

$$LAI = \frac{Leaf\ Area\ of\ Plant}{Leaf\ area\ occupied\ by\ a\ plant}$$

2. **Leaf Area Ratio (LAR):** The term leaf area ratio was suggested by Redford (1967). It is defined as the ratio of area of the leaf to the total plant biomass per plant. It is measure of leafiness or photosynthetic surface relative to respiratory mass. It is expressed in terms of $cm^2.g^{-1}$

$$LAR = \frac{Leaf\ Area\ of\ Plant}{Total\ dry\ weight\ of\ plant}$$

3. **Leaf Area Duration (LAD):** It is ability of the plant to maintain the green leaves per unit area of the land over a period of time. It reflects the vitality of leaves and an opportunity for assimilation. LAD is the integral of leaf area index over a growth period and expressed in **days**.

$$LAR = \frac{LAI_1+LAI_2}{2} x\ (t_2 - t_1)$$

Where,

LAI_1 = Leaf area index at time t_1 LAI_2 = Leaf area index at time t_2

4. **Specific Leaf Area (SLA):** Specific leaf area is the ratio of leaf area to its dry weight. It is measure of relative spread of leaves. It is expressed in $cm^2.day^{-1}$

$$SLA = \frac{\text{Leaf Area}}{\text{Leaf dry weight}}$$

5. **Specific Leaf Weight (SLW):** Specific leaf weight is the ratio of leaf dry weight to leaf area. It indicates the leaf thickness and density and it is expressed as $g.cm^{-2}$

$$SLW = \frac{\text{Leaf dry weight}}{\text{Leaf area}}$$

6. **Net Assimilation Rate (NAR):** NAR is a measure of the amount of photosynthetic product going in to plant material. It is the rate of increase of leaf by dry weight per unit area of leaf per unit time. It is expressed in $g.m^{-2}$ (leaf area). day^{-1}

$$NAR = \frac{(ln\ L_2 - ln\ L_1)\ x\ (W_2 - W_1)}{(t_2 - t_1)\ x\ (L_2 - L_1)}$$

Where, L_1 & W_1 = Leaf area and dry weight of the plant respectively at time t_1

L_2 & W_2 = Leaf area and dry weight of the plant respectively at time t_2

7. **Relative growth Rate (RGR):** It is rate of increase of dry weight per unit weight already present per unit time. Or RGR express the dry weight increase in a time interval in relation to the initial weight. It is expressed in $g.g^{-1}.day^{-1}$

$$RGR = \frac{(ln\ W_2 - ln\ W_1)}{(t_2 - t_1)}$$

Where, W_1 and W_2 are plant dry weight at time t_1 and t_2 respectively.

8. **Absolute growth Rate (AGR):** It expresses the dry weight increase per unit time and is expressed in g/plant/day.

$$AGR = \frac{(W_2 - W_1)}{(t_2 - t_1)}$$

Where, W_1 and W_2 are plant dry weight at time t_1 and t_2 respectively.

9. **Crop growth Rate (AGR):** It is the rate of increase of dry weight per unit land area per unit time. CGR is a simple and important aid of agricultural productivity. It is expressed in g. m^{-2} (land area). day^{-1}

$$AGR = \frac{(W_2 - W_1)}{(t_2 - t_1)\ x\ S}$$

Where, W_1 and W_2 are plant dry weight (g) at time t_1 and t_2 respectively.

S is land area (m^2) over which dry matter was recorded

10. **Harvest Index (HI):** It reflects the production of assimilate distribution between economic yield and total biomass yield.

$$HI\ (\%) = \frac{\text{Economic Yield}}{\text{Plant Biomass (above ground)}}\ x$$

Rough Work