## PREFACE

Crop Physiology is the basic science which caters to the need of several cognate disciplines. The present practical manual entitled 'Practical Manual on Crop Physiology' has been prepared for the students of B.Sc. (Ag) and M.Sc. (Ag) programme as per the syllabus approved by 4th Dean's committee.

To Elucidate a physiological process, one has to undertake quantative and qualitative analysis of a specific parameter. Various important physiological processes like seed germination, transpiration, plant growth and development, photosynthesis, respiration, imbibition, seed viability and vigour etc. are included in this manual. I have tried to provide appropriate solutions to the physiological problem, even with or without the most sophisticated laboratory instruments and equipments. The users of this practical manual can select suitable methods, according to the available facilities. I have made sincere efforts in presenting the procedure of various methods in simple way. All efforts have been made to provide the references also so that students can refer book and original articles for more information.

I owe a deep debt of gratitude to Dr. S.K. Patil, Hon'ble Vice-Chancellor, IGKV, Raipur for his constant encouragement and affectionate guidance. I extent my heartiest thanks to Dr. N.K. Motiramani, I/c Dean, S.K. College of Agriculture & Research Station, Kawardha (Kabirdham) for his valuable guidance, useful suggestions and unceasing interest.

With great pleasure I acknowledge the counsel, encouragement and eulogistic supports by Shri Ashish Thakre, Asstt. Prof., Shri R.S. Nag, Asstt. Prof., Shri Sunil Kumar, Scientist, Shri P.K. Netam, Asstt. Prof. and Er. D.K. Roy, Asstt. Prof., Mrs. Nisha, Asstt. Prof., Miss Kshama, Asstt. Librarian, other staff members and my family for their fruitful and endless efforts.

All necessary precautions have taken to prepare the text free of mistakes but if you come across with any of such mistakes; it would be cordially invited to improve it in future.

**Place: Kawardha:**
**Dated: September 12, 2012**

**(Vidya Bhushan Kuruwanshi)**
**Author**

छत्तीसगढ़ संवाद

# PRACTICAL MANUAL ON CROP PHYSIOLOGY




**VIDYA BHUSHAN KURUWANSHI**

*(ASSISTANT PROFESSOR)*

**Indira Gandhi Krishi Vishwavidyalaya**
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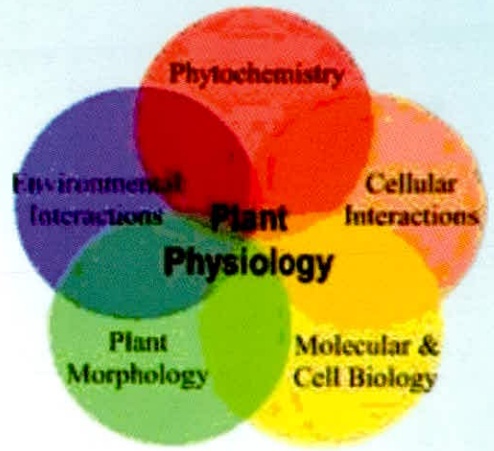
Phytochemistry
Environmental Interactions
Cellular Interactions
Plant Physiology
Plant Morphology
Molecular & Cell Biology

# PRACTICAL MANUAL

# ON

# CROP PHYSIOLOGY

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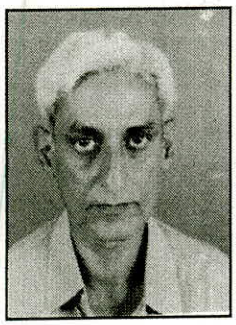
# FOREWORD



The discipline of Crop Physiology is one of the most pioneer branch of agricultural science. Several of the physiological tools and traits are commonly used in research in field of all cognate disciplines in improving crop productivity. The teaching in agricultural science need to be more practically oriented and students should be well aware of basic practical concept in translating the knowledge into action. In view of this, the publication of Practical Manual on Crop Physiology by Shri V.B. Kuruwanshi, Assistant Professor (Crop Physiology) at Sant Kabir College of Agriculture & Research Station; Kawardha is a most welcome and timely efforts to help the students.

The description have been classified in a point wise serial order and efforts have been made to collect latest information from several standard books to include all important points and details in a concise and lucid way so that slight practice on the parts of the students will considerably add to their comprehension. This handy manual is very useful for undergraduate course as well as young teachers in the field of Crop Physiology.

It gives me an immense pleasure in bringing out such a useful practical manual on behalf of Sant Kabir College of Agriculture & Research Station, Kawardha. I compliment Shri V.B. Kuruwanshi, Assistant Professor for the commendable efforts made in publishing the present manual for our College students. I hope, this manual would be extremely useful to students not only in practical but in field of teaching as well.

**(Dr. N. K. Motiramani)**
I/c Dean
Sant Kabir College of Agriculture & Research Station, Kawardha (Kabirdham)

**Place:** Kawardha
**Dated:** September 12, 2012

# *CONTENTS*

## Practical No. 01

## Object: To study the preparation of different solutions.

**Solution**

Solution is a homogenous mixture of the single phase containing one or more of the chemicals and species dispersed on the molecular scale.

**Solvent and Solute**

Solvent is a substance capable of dissolving another substance, the solute, to give rise to solution.

**Standard solution**

It is the solution, which contain a known weight of reagent in a definite volume of the solution.

**True solution**

A true solution is a homogenous solution in which the solute particle have diameter less than 10-7 i.e. the solute particles are of molecular dimensions. The particles are invisible even under powerful microscopes.

**Example:** sodium chloride in water is a true solution. Most ionic compounds form the true solutions in water.

**Molar solution**

A solution in which one gram molecular weight of the substance is dissolved in a solvent and made upto one litre in volume.

**Molal solution**

When one gram molecular weight of a soluble substance is completely dissolve in one liter of water, it makes a molal solution. It is also called 'weight molar' solution. The final volume of the solution is generally greater than one liter and this increase is volume is called the 'solution volume of the solute.'

**Percent solution**

The solution which contains known percentage of solute.

**Example:** If 5 g of solute are present in 100 ml, it is a 5% solution (w/v).

**Normal solution**

A solution that contain 1 gram equivalent weight of solute per liter of solution

**Example:** HCl react with NaOH on a 1:1 basis -1 equivalent of HCl for one equivalent of NaOH. The equivalent weight is the same as the molar weight, so the molar concentration of HCl is also same as the normality.

**Parts per million (ppm)**

Parts per million is the number of unit of mass of a contaminant per million units of total mass.

**Example:** Make a solution of 300 ppm N half of the N to be supplied by ammonium nitrate, half by Calcium nitrate ( ammonium nitrate = 33% N, Calcium nitrate = 15%)

33 x 75= 29.75

15 x 75= 11.25

150/29.75= 5

150/11.25= 13

To make up a 300 ppm nitrate solution of ½ ammonium nitrate and ½ calcium nitrate dilute 5 and 13 ounces respectively.

**Gram equivalent weight**

The weight of an element in grams which combines with or displaces from a compound 8 grams of oxygen or 1.008 grams of hydrogen or 35.5 grams of chloride.

**Gram molecular weight**

The weight of a substance in grams equal to its atomic weight units. The gram molecular weight of any substance contains of $6.02 \times 10^{23}$ molecules of that substance (Avagadro's number).

**Example:** the molecular weight of $CO_2$ is 12+32=44. the gram molecular weight of $CO_2$ is 44 grams.

## Practical No. 02

## Object: To study the Growth analysis

Growth represents overall performance of a plant under a particular environment. Generally, we restrict to the above ground plant parts for growth studies, however, a better understanding of plant growth and development would be achieved if total dry matter (including above ground shoot as well as underground root) were taken into consideration. The basic principles behind growth analysis constitutes (i) data on plant height and (ii) leaf area

Growth analysis is the method of analysing growth through plant sampling techniques. By this method plants are observed periodically for their height, leaf area, flowering time etc and also sampled at different intervals (weekly or fortnightly). When various parts of plants such as roots, stems, leaves, fruits, seeds etc are separated, weighed and measured. From these, the physiological parameters such as growth rate and relative growth rate are calculated.

### 1. Leaf Area Index (LAI)

The leaf area per unit of land area is called as leaf area index (LAI).

$$LAI = \frac{\text{Leaf area}}{\text{Ground area}}$$

LAI is a dimensionless term. Leaf area here, refers to the total surface area of the leaves, i.e. both upper and lower surfaces. Care should be taken while making comparisons between flat leaves than those of conifers. Leaf area must be measured on the same basis.

### 2. Crop Growth Rate (CGR)

The multiplication product of leaf area ratio and leaf area index gives rise to the term what we call as crop growth rate.

$$CGR = \frac{w_2 - w_1}{p\,(t_2 - t_1)}$$

Where,

$w_1$ and $w_2$ are the total dry weights at $t_1$ and $t_2$ time intervals.

p= Ground area

$$CGR = LAR \times LAI$$

It is serves as a simple index of crop productivity. It is expressed in terms of the weight per unit area and time i.e. kg $m^{-2}s^{-1}$

### 3. Relative Growth Rate (RGR)

It is defined as the amount of growing material per unit dry weight of plant per unit time.

If, $w_1$ and $w_2$ are the total dry weights at time $t_1$ and $t_2$, respectively, the mean value of RGR for time interval $t_1$-$t_2$ can be calculated as follows:

$$RGR = \frac{\log_e w_2 - \log_e w_1}{t_2 - t_1}$$

It is also called as efficiency index and can be expressed as kg $day^{-1}$.

The relative growth rate serves as fundamental measure of dry matter production and can be used to compare the performance of species or the effects of treatments under strictly defined conditions.

### 4. Net Assimilation Rate (NAR)

Net assimilation rate (NAR) can simply be defined as increase in dry weight of plant per unit leaf area per unit time.

$$NAR = \frac{(w_2-w_1)(\log_e l_2 - \log_e l_1)}{(t_2 - t_1)(l_2-l_1)}$$

Where,

$l_1$ and $l_2$ are total leaf area at a times $t_1$ and $t_2$ respectively.

$w_1$ and $w_2$ are dry weights during the same period. It is expressed as kg $m^{-2}d^{-1}$

The expression gives an accurate estimation of NAR only if the relationship between l and w is linear over a period of time.

### 5. Leaf Area Ratio (LAR)

It is the ratio of leaf area to dry weight of plant. It can be calculated by the following equation:

$$LAR = \frac{l_2 - l_1}{(\log_e l_2 - \log_e l_1)} \times \frac{(\log_e w_2 - \log_e w_1)}{w_2 - w_1}$$

Where,

$l_1$ and $l_2$ are total leaf area at time $t_1$ and $t_2$ respectively.

$w_1$ and $w_2$ are dry weights during the same period.

### 6. Specific Leaf Area (SLA)

It is define as the leaf area per unit leaf weight.

$$SLA = \frac{\text{Leaf area}}{\text{Leaf weight}}$$

It is expressed as $m^2$ $kg^{-1}$

**7. Specific Leaf Weight (SLW)**

It is just reverse of specific leaf area.

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

It is expressed as kg $m^{-2}$

SLA defines leaf area ratio in terms of leaf density and its reciprocal (SLW) is a measure of the leafiness of plant on a weight basis.

This is an indirect measurement of leaf photosynthetic rate and leaf expansion under diverse environment and management conditions.

**8. Leaf Area Duration (LAD)**

It is the measure of persistence of assimilatory surface. There is no instantaneous value of D and it is normally calculated from the relationship between leaf area index and time. It is determine from a plot of leaf area index against time.

$$\text{LAD (on Leaf area basis)} = \frac{(LA_1+LA_2)\,(t_2 - t_1)}{2}$$

$$\text{LAD (on Leaf area index basis)} = \frac{(LAI_1+LAI_2)\,(t_2 - t_1)}{2}$$

It is the product of a dimensionless unit and time. The unit of leaf area duration is time (usually expressed in days).

**9. Harvest Index (HI)**

It is also called as "Co-efficient of effectiveness of formation of economic part of the total yield".

The three parameters namely- economic yield, biological yield and harvest index are related by the following equation:

$$HI\ (\%) = \frac{\text{Economic yield}}{\text{Biological yield}} \times 100$$

Harvest index is obtained by dividing the economic yield with the biological yield.

## Practical No. 03

## Object: To measurement of Below Ground Biomass.

Measurement of root biomass is equally important as the shoot biomass. By measuring the shoot biomass alone it would be pretty difficult to answer whether the apparent increase above the ground productivity is due to photosynthetic grain or simply because of re-distribution of matter from the underground root.

The entire process of root biomass study is accomplished in the following steps:

**1. Extraction of Root**

Root samples are taken from the centre of the plot clipped for shoot sample studies. Generally, we use (a) trenches or (b) cores to take below ground samples, depending upon the soil type and soil water availability.

**2. Washing**

Roots should be completely washed of the clay, silt, sand and organic matter. A simple root washing (Fig.03a) machine consists of a long cylinder (area = 1000 $m^3$) centrally fitted with a plunger having a perforated circular base. The plunger moves vertically and disperses the soil sample. Roots and organic matter are then decanted off.

A vortex root washer (Fig.03b) may also be used for this purpose. In the vortex washer water flows with speed and through the out flow roots and organic matter fall on the sieve band. Sand and larger particles fall to the base of the washer while clay and silt are passed through the sieve.

Iron sulphide deposits on roots (in case of waterlogged soil) can be removed by placing the washed root in continuously aerated water for 24-48 hrs.

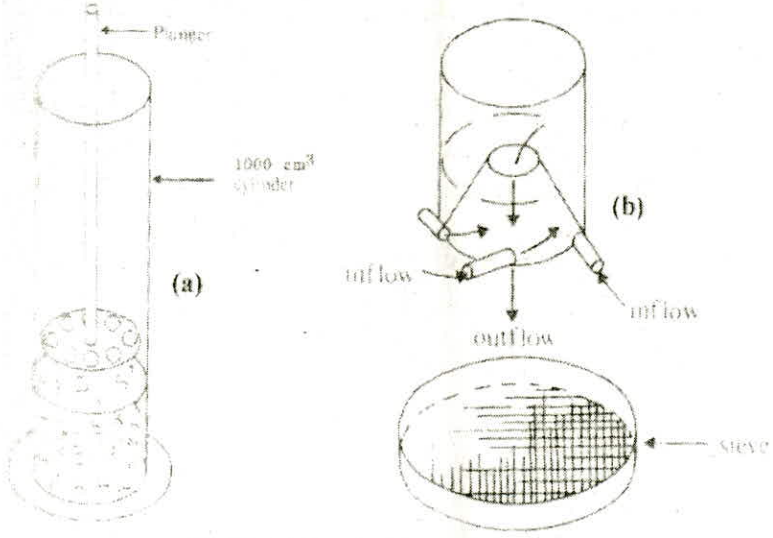



**Fig 03: (a) Simple (b) Vortex root washer**

**3. Removal of Dead Material**

The roots, extracted and washed as above, should be separated from the decaying and dead matter (Hussey and long, 1982). For this purpose, the extracted roots are put in solvents such as methanol or hydrogen peroxide. The living root material floats and dead root material sinks on the bottom of the container.

**4. Measurement of Dry Weight**

Measurement of dry weight of root is exactly the same as the above ground mass or the shoot. Determination of weight loss on ignition is particularly valuable for below ground biomass, since this eliminates contamination by inorganic soil mineral particles.

**Reference**

Hussey, A. and Long, S.P. (1982). Seasonal changes in weight and above and below-ground vegetation and dead plant material in a salt marsh at clone point, Essex. *j. Ecol.* **70**:752-772.

## Practical No. 04

## Object: Measurement of Relative Water Content of leaf.

### Principle

Plant leaves generally have lower (more negative) water potential than pure water; hence, they osmotically absorb water and become turgid. A measure of this property is the Relative Water Content (RWC) which expresses the leaf water content (%) of the turgid leaf water content. It is calculated by the formula:

$$\text{RWC (\%)} = \frac{\text{Fresh weight - Oven dry weight}}{\text{Turgid weight - Oven dry weight}} \times 100$$

As similar parameter is the Water Saturation Deficit (WSD) that is computed by the formula:

$$\text{WSD (\%)} = \frac{\text{Turgid weight- Fresh weight}}{\text{Turgid weight- Oven dry weight}} \times 100$$

The water is absorbed till the existence of a Water Saturation Deficit (WSD) (i.e. till the tissue becomes fully turgid and stops absorbing water) so that RWC + WSD = 100% or WSD = 100- RWC (Kramer, 1983).

### Material required

Leaf tissue, deioniesed water, Petri dishes, balance etc.

### Procedure

Excise the leaf tissue (or any other tissue) whose RWC is to be determined. Weigh the tissue and now dip the tissue in deionised water in covered Petri dishes or any other suitable container for 4-5 hrs. at room temperature till the tissue is fully saturated in a perfectly humid environment. Remove the tissue, blot it to remove surface water and weigh to get the turgid weight. Now dry the tissue to a constant weight in an oven at 65-70$^0$c for 72 hrs. and weigh it to record the dry weight.

### Interpretation of results

Determine the RWC from the recorded parameters (Table.1) as described as above:

**Table.1: Calculation of relative water content (RWC)**

| Tissue | Fresh weight (g) | Turgid wt. (g) | Dry weight (g) | RWC % |
|---|---|---|---|---|
| 1.<br>2.<br>3. | | | | |

**Precautions**

1. For fresh weight, weight the tissue immediately.
2. For turgid weight, tissue should be kept dipped in water for sufficient time (4-6 hrs.) till constant weight is obtained.
3. If leaf tissue is too large, then leaf discs can be taken.

**Reference**

Kramer, P.J. (1983). *Water relations of plants*. Academic Press, Orlando, USA. pp. 1-665.

## Practical No. 05

## Object: Measurement of water potential of plant tissue by Chardakov's method.

This method is developed by Chardakov's a Russian scientist, can be used in the laboratory as well as the field and does not required any sophisticated instrumentation.

**Principle**

Concentration of the solution in which a plant tissue is immersed changes (increases or decreases), depending upon the water potential of the tissue and the solution. If the solution is hypertonic (with lower ψw) the tissue losses water and the solution becomes dilute. If the solution is hypotonic (with higher ψw) the tissue absorbs water and solution becomes concentrated. If the solution is isotonic (with lower ψw equal to the tissue sap), the tissue neither losses nor absorbs water and the solution concentration does not change. Thus the ψw of this solution gives the ψw of the tissue (Salisbury and Ross, 1969).

**Material required**

Leaves or any other tissue of the plant to be tested, test tubes, sucrose or polyethylene glycol (PEG) 6000 solution, methylene blue, dropper.

**Procedure**

Prepare graded concentrations of sucrose or PEG 6000 of different molalities i.e., 0.05, 0.1, 0.15, 0.2, 0.25, 0.3 (Fig 3.1). Take these solutions in two test tubes each. Colour one set of each solution by dissolving a small crystal of methylene blue in it (control solution). This does not change the ψw of the control solution than to its non-coloured counterpart (test solution). Immerse tissue samples (approximately equal size) in each colourless test solution test tube (fig. 05). Allow the tissue to come into equilibrium with the solution for about 15-20 min. at room temperature. Remove the tissue from the test tubes. Put a drop of the corresponding coloured control solution in the colourless test solution in the center. Record the movement of the drop i.e., whether it rises up, diffuses or sinks.

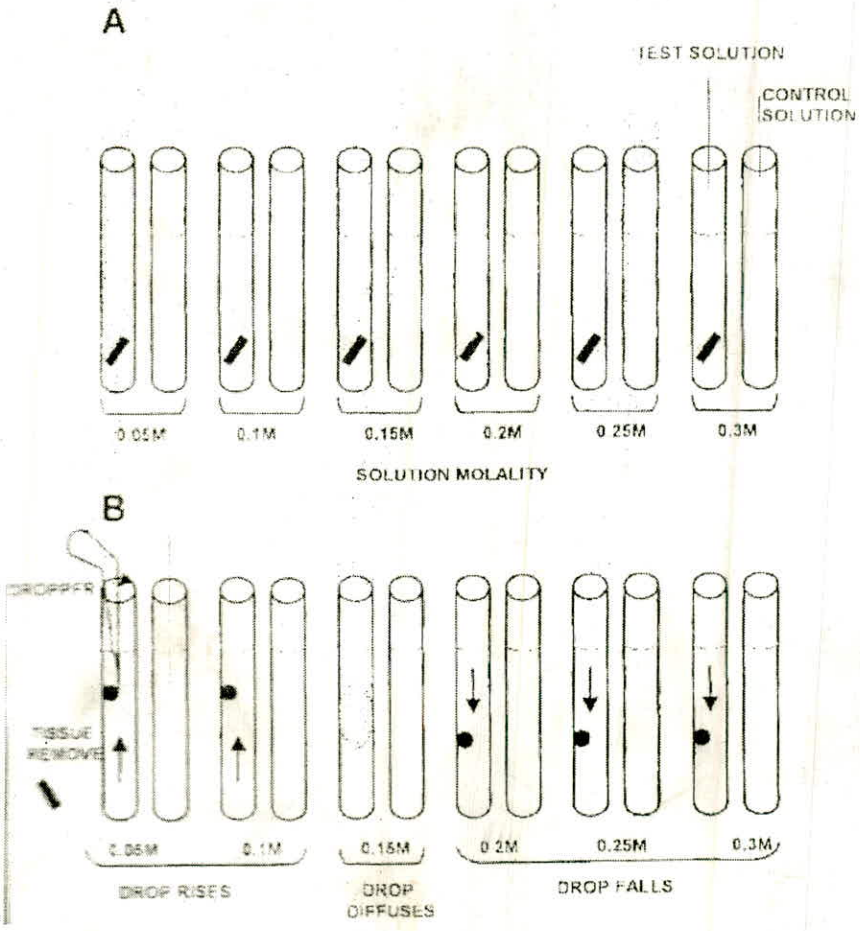



**Fig 05: Measurement of water potential of plant tissue by Chardakov's method.**

**Interpretation of results:**

If the control solution drop rises up the test solution, in which the tissue was incubated has become dense due to absorption of water by the tissue. If the drop sinks test solution has become less dense, having osmotically absorbed water from the tissue. If the drop diffuses evenly into the test solution without rising or sinking, then no change in the concentration has occurred and the water potential of the solution equals to that of the tissue. Chardakov's method is very simple and fairly sensitive for determination of plant tissues even in the field.

**Precautions**

1. To avoid evaporative losses, the test tissue should be immediately dipped in the graded solutions and solutions in the test tubes should be kept covered with glass marbles.
2. Tissue size does not affect the end result, but it should be of nearly uniform size/thickness to allow uniform equilibration time.

3. Allow sufficient time for tissue to equilibrate with the test solution. For non-waxy leaves 4-5 hrs time is sufficient.

4. Put the drop of control solution into test solution very gently.

The movement of control solution drop in test solution should be recorded in following (Table 02).

**Table 02.** The movement of control solution drops in test solution.

| S.N. | Molality of solution | Direction of drop movement* |
|---|---|---|
| 1. | 0.05 | |
| 2. | 0.10 | |
| 3. | 0.15 | |
| 4. | 0.20 | |
| 5. | 0.25 | |
| 6. | 0.30 | |
| 7. | 0.35 | |

* Rises up, diffuses, sinks

**Reference:**

Salisbury, F.B. and Roŝs, C. (1969). Osmosis and component of water potential. In *Plant Physiology*, Prentice Hall of India, New Delhi, pp. 52-74

## Practical No. 06

## Object: Estimation of Chlorophyll

Chlorophyll can be regarded as true representation of health of green plants. This presumption is based on the face that chlorophyll is the most commonly and immensely influenced by biotic and abiotic stress conditions in addition to events like pathogenic invasion, pollution, mineral deficiencies as well as mineral toxicity. This is the reason that estimation of chlorophyll is considered to be one of the most important parameters in plant physiological studies.

**(a) Acetone method (Arnon, 1949)**

Chlorophyll is soluble in acetone. When the sample is macerated in acetone, chlorophyll gets dissolved in it. The optical density of the extract is measured at 663 and 645 nm wavelengths using a spectrophotometer or colorimeter because at these wavelengths, maximum absorption of chlorophyll 'a' and 'b' takes place, respectively. The amount of chlorophyll 'a', 'b' and total chlorophyll are determined using the formulae given by Arnon (1949).

Chlorophyll 'a' = $[(12.7 \times A_{663}) - (2.69 \times A_{645})] \times V/1000 \times W$

Chlorophyll 'b' = $[(22.9 \times A_{645}) - (4.68 \times A_{663})] \times V/1000 \times W$

Total Chlorophyll = $[(8.02 \times A_{663}) + (20.2 \times A_{645})] \times V/1000 \times W$

The value, so obtained, are expressed as mg chlorophyll per gram fresh weight of sample, where;

A = Absorption at given wavelength,

V = Total volume of sample in extraction medium and

W = Weight of sample (g)

**Material Required**

(i) Acetone (80%), (ii) Pestle and Mortar, (iii) Funnel, (iv) Whatman Filter Paper No.1, (v) Volumetric flasks (25 ml), (vi) Measuring cylinders (50, 100 ml), (vii) Pipettes, (viii) Test tubes, (ix) Spectrophotometer/Colorimeter, (x) Microbalance, (xi) Plant material.

**Procedure**

Take 200 mg freshly cut material
(leaf pieces devoid of mid rib)

↓

Grind in a pestle with mortar (use 10 ml 80% acetone)

↓

Filter the homogenate in a volumetric flask (25 ml)
(using Whatman Filter Paper No.1)

↓

Washout the homogenate 2-3 times with 5 ml of 80% acetone each time

↓

Make the final volume of the filtrate up to 25 ml with 80% acetone

↓

Take the filtrate in a cuvette (3/4 volume) and record its absorbance at 663 and 645 nm separately using Spectrophotometer/ Colorimeter (keep 80% acetone as blank)

↓

Calculate the amount of chlorophyll (with help of formulae given below)

**Precautions**

(i) Extraction should be carried out at subdued light and low temperature because light influences pigments.

(ii) 2-3 washings are necessary to ensure maximum extraction of chlorophyll pigments.

**(b) By SPAD or Chlorophyll Meter**

It is a sample and non-destructive method of chlorophyll estimation. It can be used in the laboratory as well as in the field of standing crop.

**Principle**

SPAD (Soil and Plant Analytical Development) is a simple diagnostic tool (manufactured by M/s Minolta, USA) (Fig. 06). We get readings in terms of SPAD values that indicate relative chlorophyll content. It is based upon an unique linear relationship between SPAD and leaf area based nitrogen concentration in plants (Peng *et al.* 1993). However, it requires standardization/calibration for each crop because this relationship varies with crop growth stage and variety.

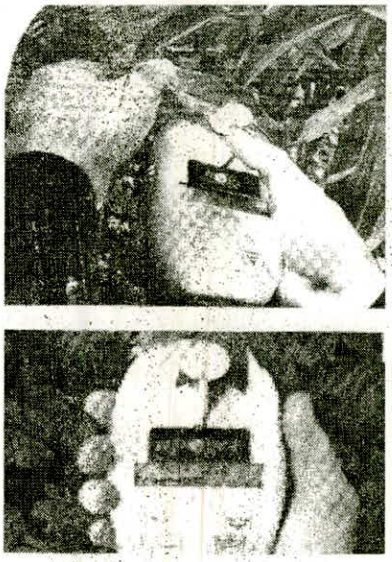


**Fig 06: SPAD or Chlorophyll meter**

**Procedure**

(i) Switch on the instrument and let it warm up for about 10-15 minutes.

(ii) Calibrate the instrument for accuracy check using a special disc provided with the instrument.

(iii) As soon as the 'beep' sound is over, put a fully expanded leaf into it in such a way that only the leaf portion between the lamina and the mid-rib remains inside.

(iv) Note down the reading. To check a reading, take another reading on the same leaf.

(v) Use 'average' button to calculate the average of readings taken automatically.

(vi) Close until you hear 'beep'.

**Precautions**

(i) If you want to take reading of a detached leaf in the laboratory, care should be taken so that leaf does not wilt.

(ii) While working in the field, you should shield the leaf (sample) from direct sunlight.

**References**

Arnon, D.I. (1949). Copper enzymes in isolated chloroplasts: Polyphenol oxidases in *Beta vulgaris*. *Plant Physiology*. 24:1-14.

Peng, S., Garcia, F.C., Laza, R.C. and Cassman, K.G. (1993). Adjustment for specific leaf weight improves chlorophyll meter's estimation of rice leaf nitrogen concentration. *Agron. J.* 85: 987-990.

## Practical No. 07

## Object: Measurement of leaf area by various methods.

Estimation of the area of leaves and the surface of other assimilating plant organs is an essential part of classical growth analysis and is necessary in many plant physiological studies. Several principles are applied to determine the leaf area. The choice of methods depends on the main purpose of measurements i.e.

(a) Whether the total leaf area or (b) Individual leaves (c) It should be destructive or (d) Non-destructive. Some important methods are described below:

**A) Leaf outline on graph paper**

In this method the leaf area of the plant is measured by using the graph paper and grid on the acrylic plastic sheet. The method is very economical and can be used without the electricity/power in field conditions.

**Material required**

Leaf sample, graph, acrylic plastic sheet etc.

**Procedure**

1. Place the leaf on graph paper or grid squares made on transparent acrylic plastic sheet for calculating the area blocked by leaf in sq cm and outline its margin.
2. Obtain the area by counting the squares within the outline
3. In field conditions, mark a grid on a clear piece of acrylic plastic sheet, against which the leaf is held, and count the square blocked out by the leaf.

**Advantage**

This method is fairly accurate.

**Disadvantage**

1. The accuracy of this method depends on the leaf shape, for highly divided or compound leaves, this types of leaf area measurement is impractical.
2. It requires long time and difficult to record leaf area of large number of leaves.

**B) Leaf weight method**

This method depends upon the leaf area: Leaf weight ratio and is used in growth analysis of plant stands.

**The leaf area:** leaf weight is estimated in a representative sub sample, using any methods of the leaf area measurement. The relationship between leaf area and leaf fresh weight is used in determine the leaf area (Turrell, 1961).

**The variations in leaf area :** leaf weight ratio, taking leaf fresh weight, air dry weight or oven dry weight was prevented by Alenkseenko (1965).

**Relationship between leaf area and leaf water content**

Leaf area is linearly related to absolute leaf water content (leaf fresh weight – leaf dry weight) when

$$A = a + bw$$

Where,

A is total leaf area

w is the absolute leaf water content

a and b are the constant

**C) Measurement of leaf area by leaf area meter**

This portable leaf area meter (Fig.07) provides non-destructive, precise method for monitoring crop canopy development or individual plant growth in the field or growth chamber. Leaf area reduction and recovery associated with condition such as insect infestation, air pollution, drought and disease can be evaluated by measuring the same plant throughout the season.

**Principle**

Leaf area mater uses electronic methods to stimulate a grid pattern on the leaf. The scanning head uses a row of 128 narrow bands, red light emitting diodes (LEDs), spaced of one mm centers, to examine 128 grid cells across the width of leaf. The LEDs are sequentially pulsed (only one LED is lit at a time) to examine a particular grid cell in the row. The LEDs are located along a line 0.62cm from the edge of the upper section of the scanning head. The base of the scanning head contains Lense- Photodiode system, respond only to collimated, pulsed LED light. This design makes the measurement insensitive to other light sources.

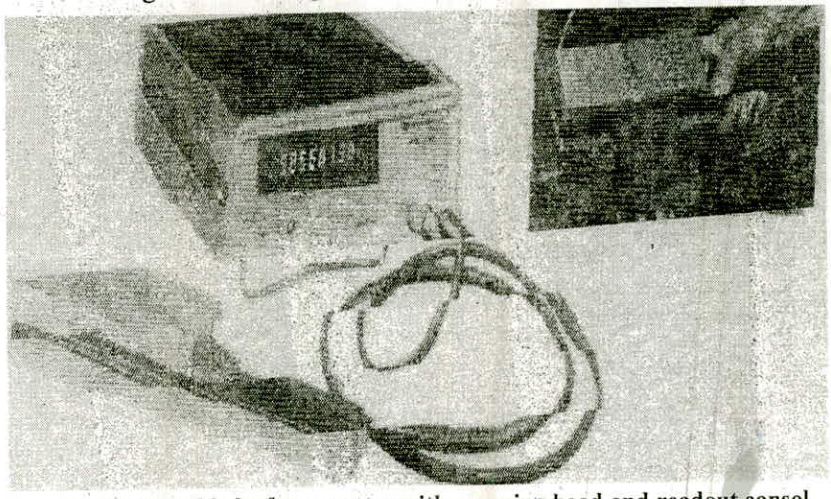


**Fig 07: Portable leaf area meter with scanning head and readout consol**

**Material required**

Portable leaf area meter have two components, the scanning head and readout consol. The consol can also be used with transparent belt conveyor, to determine leaf area of detached leaf sample.

**Procedure**

1. Connect the scanning head to the readout consol.
2. Turn the ON-OFF switch to ON.
3. Open the scanning and position it over a leaf. The leaf does not need to detached from the plant.
4. Close the scanning over the petiole, so that the leaf base is in the scanning head but not between the LED and photodiode windows.
5. Draw the length encoding cord to some location, where it can be held steady. The knob can be held against a stem or in broad leaf plants. The knob can be hold firm, by clasping it together with the petiole between the second and third fingers.
6. Press the scanning head button, to clear any reading registered before starting the measurements.
7. Draw the closed scanning head over leaf, while the length encoding knob is held stationary. Drawing speed needs to be constant.
8. After the scanning head has passed over the leaf. Let the length encoding cord slowly rewind itself back into the scanning head.
9. Note down the reading (sq cm) on the consol unit.

**References**

Alekeenko, L.N. (1965). Weight method of leaf area determination of meadow plant and communities. *Botanical Journal* **50**: 205-208.

Turrell, F.M. (1961). Growth of photosynthetic area of citrus. *Botanical Gazette* **122**: 284-298.

## Practical No. 08

## Object: Estimation of Stomatal Frequency and Stomatal index with Replica Method.

Wolf and his associates (1979) developed this rapid simple and suitable method and can be employed in most of the crop plants which are non waxy and non hairy. Blue/red correction fluid or transparent figure nail polish (collodion) is used as replicating material. Fluid-dipped brush is stroked across the area to be replicated with a single motion. After few seconds when fluid become firm, the replica can be removed. A small piece of celluloid tape is secured to a glass slide. The exposed sticky surface is then placed over the replica and with slight pressure the film is removed from the leaf surface. The replica is mounted for viewing as an imprint on reserve side of the image of leaf surface. Using microscope with pre-calibrated grid, the number of stomata and epidermis cell can be counted.

The **stomatal index (SI)** can be calculated as follows:

1. Take a circular piece of paper of the size of eyepiece with a rectangular cut in the center. Insert the paper in an eyepiece.
2. Count the number of epidermal cell (E) and stomata(S) with in the rectangular area.
3. Plug in the value in the following equation and get the values of stomatal index.

$$SI\ (\%) = \frac{S}{E+S} \times 100$$

S= Number of stomata per unit area

E= Number of epidermal cells per unit area.

**Stomatal frequency (*f*)** can be calculated as follows:

1. Count the number of stomata within the area of rectangle/square. Half cut stomata is also counted.
2. After knowing the scanned area and the stomatal number *f* can be calculated in $mm^2$ per unit leaf area.

## Practical No. 09

## Object: Measurement of Respiration rate.

Respiration rate, irrespective of plant parts (*viz.* leaves, fruits or seeds), is considered to be an important parameter in plant physiological studies. Respiratory gas exchange is just reserve on opposite to that of photosynthesis and as the later is an energy building phenomenon where ATP is stored in the photosynthates produced; respiration involves breakdown of photosynthates for release of energy to be utilized for the performance of other activities of life. Respiration plays an important role during seed germination. A germinating seed requires a lot of energy which is derived from the breakdown of different types of food reserved in the cotyledons. On the basis of respiration a climacteric fruit can well be distinguished from a non climacteric one; as the former reveals a characteristic 'Climacteric Bust' in respiration, which is completely lacking in the later.

There are few instruments used for the measurement of respiration in plants viz.

### A. Infra-Red Gas Analyzer (M/S ADC, England ;M/s LI-COR,USA)

It is generally used for the measurement of photosynthesis. However, it can also be used for measuring respiration provided:

1. The sample chamber (containing leaves) is covered with dark black cloths; so that no light reaches the leaf surface and photosynthesis may be avoided; and
2. Necessary tubing connections are made depending upon the 'closed' or 'open' type of measurement mode, as required.

Here, we measure the rate of evolution of $CO_2$, Which is taken to be the rate of respiration.

### B. Respirometer (M/s pp systems, UK)

Basically, it is an Infra-Red Gas Analyzer, however, its $CO_2$ measurement range varies from 0-50,000 ppm ($\mu mol\ mol^{-1}$) or even higher. Hence, it can be conveniently used for the measurement of respiration of large amount of sample such as fruits of big size etc. Secondly, this instrument is equipped with an 'Auto Zero' device which allows for fast warm up, adaptation to changing ambient conditions and excellent stability for the $CO_2$ signal.

### Principle

Gases with di-atomic molecules such as $CO_2$ strongly absorb photon in the infra-red range. For $CO_2$, one region of strong absorption is 4.26 microns. A source emitting strongly at this wavelength is a light bulb. If this is positioned at one end of a tube and at the other end is placed a sensor that is sensitive to photon at 4.26 micron, we have a simple infra red

analyzer. As carbon dioxide is passed down the sample cell, it absorbs some of the infra-red radiation and the sensor reading decreases.

**Respirometer (Fig.09)**

It consists of the following parts:

- Electronic housing with air supply fan
- Air supply chimney
- Sample chamber; and
- Environmental Gas Monitor for $CO_2$ (EGM-4)

There are two gas ports on the top of the EGM-4; 'Gas in' and 'Gas out'. The sampling line is fitted to 'Gas in' and 'Gas out' line is left open to atmosphere to allow the sample air to exhaust without restriction **(open system)**. For **'closed system'**, one gas line of the sample chamber is connected to the 'Gas in' port and the other to the 'Gas out' port.

On the rear panel, there is an absorber column. It contains soda lime. When air passes through this column, it removes all of the $CO_2$ from the air stream. The 'Auto Zero' facility, built into the EGM-4, periodically switches the flow of the gas from the analyzer through this column to check the analyzer zero. This routine ensures long term stability and accuracy of the $CO_2$ analyzer. Soda lime turns green to brown when exhausted. It should be removed with fresh stock periodically.

The EMG-4 has one connector used for transferring stored data to a PC or Laptop computer. It has a 2x16 character backlit LCD display. The keypad consists of 12 keys, each having specific function. The system is supplied with an AC adaptor/ charger for charging the internal 12V lead acid battery.

**Assembly**

The air supply chimney is pushed into the air supply fan and a 3-pin electrical connector (from the Mass Flow Meter) is connected to the Electronic Housing.

The exhaust pipe is attached to the sample chamber and the later is pushed on to the port next to the air supply fan. The sample chamber is also connected with the Electronic Housing using a 6-pin connector. The electronic housing is finally connected with the EGM-4.

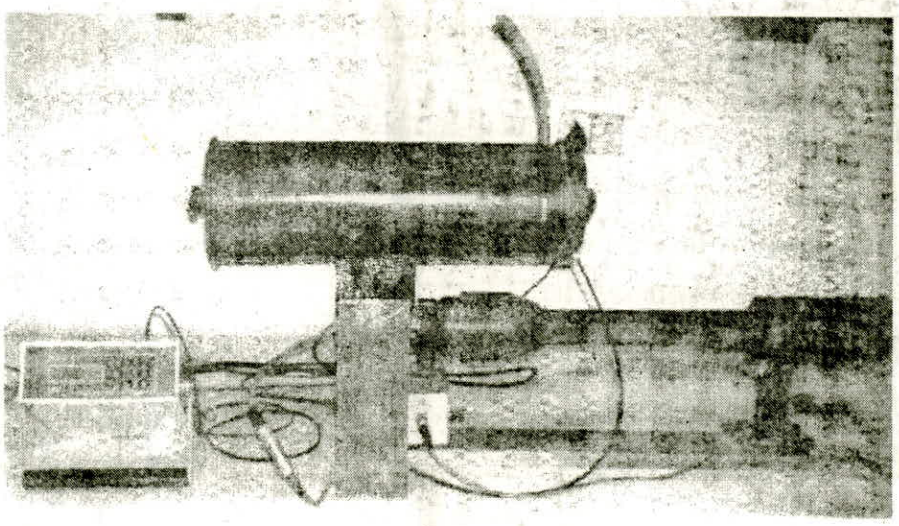


**Fig 09: Respirometer**

**Operation**

Air is pushed through the sample chamber by the air supply fan and flow rate is controlled by the mass flow meter. Rotating the flow control knob on the electronic housing, the flow rate in the chamber is adjusted.

The air flow rate should be adjusted to give 20-30 ppm differentials. Thereafter.

Switch on the instrument

↓

Go to main menu

↓

Wait for 10 minutes (warming up)

↓

Press '1'

↓

Press '2'

↓

Press 'Y'

↓

Wait for 5 minutes (auto zero)

↓

Press 'X'

Put the sample in the chamber and cover the lid

↓

Start measurement

The abbreviated form of digital output appears as follows:

Rnnnnn Dnnnn Tnn

A+/- nnnn Fnnnnn

*Where,*

R = Reference $CO_2$

D = Differential $CO_2$

T = Chamber temperature

A = Assimilation rate

F = Flow rate (ml/min.)

Measurement is automatically corrected for temperature and pressure. Results are given in μmol $m^{-2}$ $s^{-1}$ for $CO_2$ To convert it into g $CO_2$ $m^{-2}$ $h^{-1}$, the values should be multiplied by 0.1584.

**Reference**

EMG-4 Environmental Gas Monitor for $CO_2$: Operators Manual (2000). M/s PP Systems, Inc.241, Winter Street, Haverhill, MA 01830, USA

## Practical No. 10

## Object: To study the leaf anatomy of $C_3$ and $C_4$ plants.

**$C_3$ plants leaf anatomy** (Fig.10a)

- The bundles sheath cells do not contain chloroplasts; carbon fixation and Calvin cycle reactions occur in mesophyll cells in the presence of oxygen.
- More adapted to environments with more carbon dioxide.

**Examples: Wheat, Barley, Sugarbeet etc.**

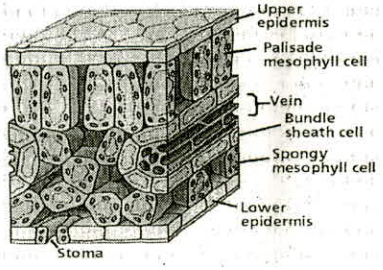
**$C_4$ plants leaf anatomy** (Fig.10b)

- The vascular bundles of $C_4$ leaves have large Photosynthetic Bundle sheath cells.
- One to three layers of Photosynthetic Mesophyll cells that surround the Bundle sheath and radiate away from the Bundle Sheath. These resemble a Wreath and this has been called **Kranz (wreath) anatomy**.
- The Bundle sheath cells contain chloroplast; carbon is fixed in mesophyll cells too, then transported to Bundle sheath cells where Calvin cycle reaction occur in the absence of oxygen.
- More adapted to environments with more oxygen.

**Examples: Sugarcane, Maize etc.**

In both, photosynthesized sugars then enter the plants vascular system.

**Fig 10(a): Leaf anatomy of $C_3$ leaves.**



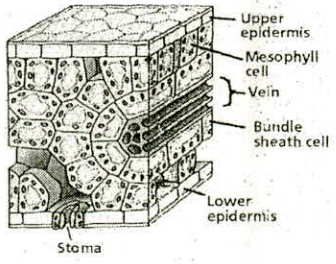




**Fig 10 (b): Leaf anatomy of $C_4$ leaves.**

## Practical No. 11

**Object: To demonstrate the development of imbibitional pressure by germinating seeds.**

### Imbibition

The adsorption of water by hydrophilic colloids is known as imbibition. Imbibition of water increases the volume of the imbibant due to which pressure is created which is known as imbibitional pressure.

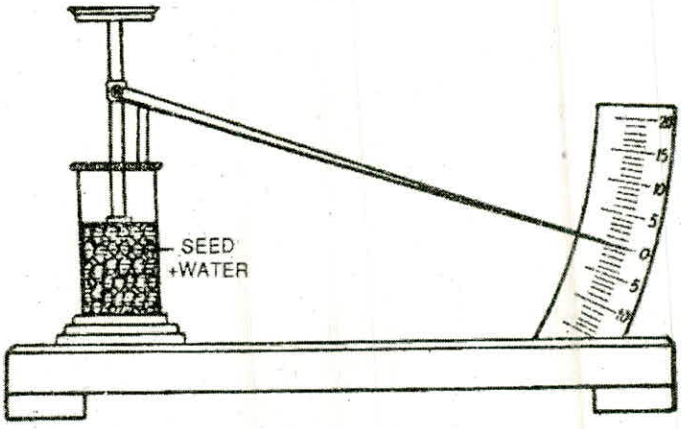



**Fig. 11: Apparatus to measure the imbibitional pressure .**

### Procedure

The apparatus (Fig.11) consist of a bottle which contains a vertical metallic rod with round discs at the two ends.

A few gram (*Cicer spp.*) seeds in water are placed in a bottle and disc just above the seed level fitted through the cork of bottle. The pointer is adjusted at zero on the scale and the apparatus is made airtight and is left for a few hours (may be up to 24 hours).

### Observation

It observes that disc is displaced above and the pointer moves down. It happens because gram seeds imbibe the water and swell considerably. The imbibition is caused due to hydrophilic colloids present in the gram seeds. The pressure which is responsible to push the disc is called imbibitional pressure.

## Practical No. 12

## Object: To Demonstrate the measurement of Transpiration.

Stephan hales (1727) in his *vegetable staticks* first describe a method of demonstration transpiration in plants. The leaves of a well watered plant were closed in transparent bell jar and water vapours condensing on the inner sides of the jar were seen. Hales devised a variety of method for measuring the rate of transpiration. Some of the methods are described here:

**A) By weighing potted plants**

A potted herbaceous plant is taken. It is well watered and the pot including soil surface is well covered with a waterproof material to prevent evaporation of soil. The pot is kept over a top pan balance and loss of weight over a definite time is noted (Fig.12A). It will be reasonable to employ this method of transpiration measurement over short periods only. If the measurements are continued for a few days, the increase in plant weight may occur due to growth.

**B) By potometer**

With potometers (meaning drink measurer), the rate of water loss from a cut plant or shoot can be measured assuming that the rate of water absorption is equal to the rate of transpiration. There are several types of potometers. In one type, there is a water reservoir to which the plant or its parts are fitted (Fig.12B). To this reservoir a glass capillary tube of known diameter is attached. A scale is also attached to the glass tube. An air bubble is introduced in the capillary tube and its movement is recorded with the help of scale. From the diameter of the tube, the amount water absorbed (hence amount transpired) can be calculated.

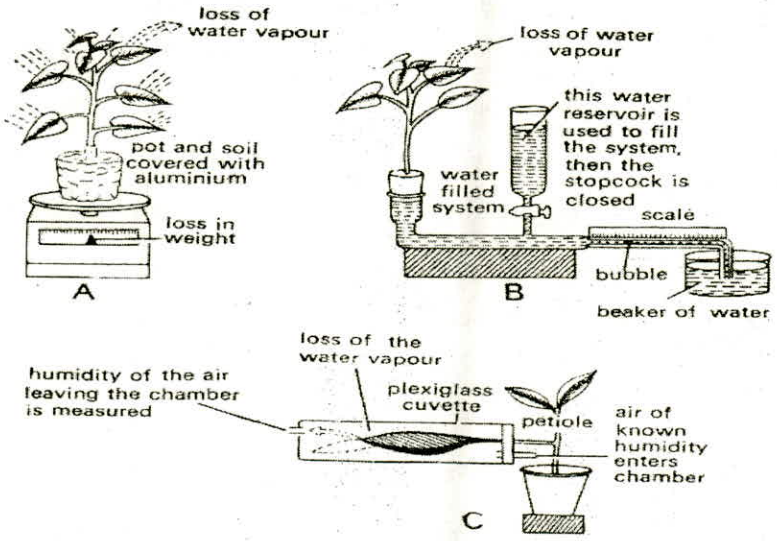



**Fig 12.(A): By weighing a plotted plant (B) By a potometer (C) By measuring humidity in the air**

**C) By measuring the humidity of air**

This method can be used for measuring the rate of transpiration from single leaf. The leaf is enclosed in a Plexiglass (plastic glass) chamber and sealed. Air is passed through the chamber at a definite flow rate. The humidity of the air before entering the chamber and after leaving the chamber is measured (Fig.12C). The amount of water transpired can be calculated by finding out the difference in humidity. Different methods are available for measuring the humidity of the air. In a simpler method, humidity is measured by collecting it through hygroscopic substances such as $P_2O_5$ or $CaCl_2$. The attached leaves are enclosed in the chamber which is also contains small tubes containing weighed amount of $P_2O_5$ or $CaCl_2$. In a control chamber, similar tubes are placed but no leaf is enclosed. The tubes are weighed after a definite time and the amount of water transpired by leaf is calculated.

## Practical No. 13

## Object: To study of the germination of seeds.

**Seed germination**

As per International Seed Testing Association (ISTA), "The emergence from the embryo and development of those essential structures, which indicate the ability of seed type tested to develop into normal plant under favorable environment."

**OR**

An agricultural point of view, germination may be defined as a process, which begins when the dry seed is planted in moist soil and ends when the seedling emergence above ground.

**Types of seed germination**

Germination has been classified into the following types:

**(A) Hypogeal germination**

In hypogeal germination (Fig.13B), the cotyledons or comparable storage organs do not emerge above the soils surface; only the plumule emerges above the ground. This is because in this type germination the epicotyl (plumule), and not the hypocotyl, undergoes rapid elongation.

**Example:** Corn, Peas, Grass, Maize seeds etc.

**(B) Epigeal germination**

In epigeal germination (Fig.13A), the cotyledons or comparable storage structures emerge above the soil surface. Thus, in contrast to hypogeal germination, there is rapid elongation of hypocotyl, which pushes the cotyledons and the plumule enclosed within them through the soil.

**Examples:** Bean, Pine seeds etc.

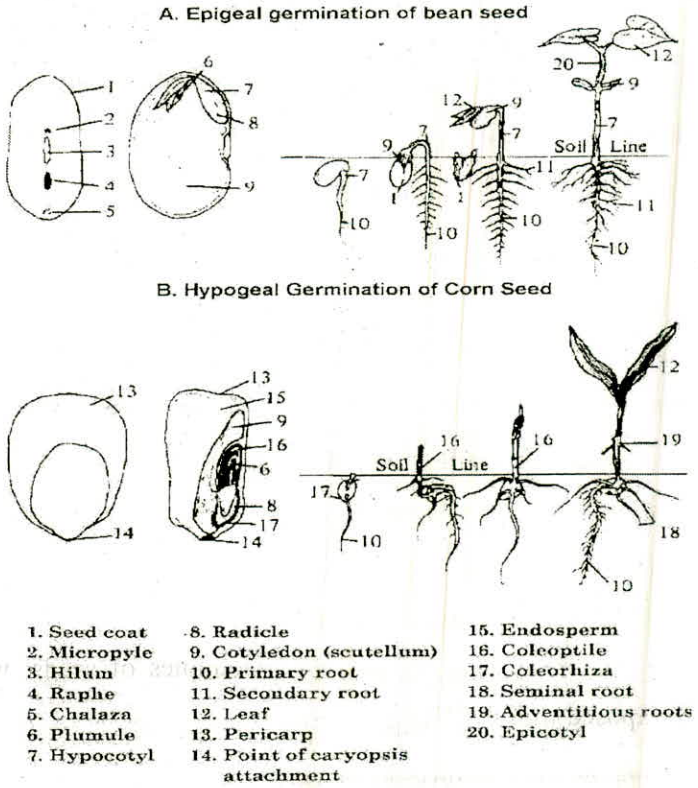



**Fig13: (A) Epigeal germination of corn seed (B) Hypogeal germination of bean seed**

## Optimum conditions for seed germination

Water, suitable temperature and oxygen are essential requirements for the germination of seed. Seeds of some species, however, require a fourth factor i.e. light.

### Water

Water is absorbed through the micropyle and seed coat. It is of paramount importance for the germination of seed. Water brings about the following changes in the germination seed:

(a) the hydration of the seed coat increases its permeability to oxygen and carbon dioxide.

(b) The swelling caused by the absorbed water ruptures the seed coat. The facilitates the entry of oxygen and the release of accumulated $co_2$. Rupture of the seed coat also helps in the emergence of embryo.

(c) The enzymatic hydrolyses of different kinds of organic food take place in the presence of water.

(d) Water provides a medium for translocation of soluble substances.

(e) The respiratory breakdown of complex organic compounds takes place in the presence of water.

**Oxygen**

Aeration of the soil is absolutely essential for the germination of the seed. Seeds which lie buried in the deeper layers of the soil fail to germinate due to lake of oxygen. Oxygen is necessary for the aerobic respiration by which the seeds get the requisite energy for the growth of the embryo.

**Temperature**

Dry seeds can withstand large extremes of temperature. A water soaked seed, however, is very sensitive to temperature variations and therefore, seeds germinate within a normal range of temperature. The minimum, optimum and maximum values of temperature for the germination of seed vary from habitat to habitat and species to species.

**Light**

Light is not indispensable for the germination of seeds. Seeds can germinate well even in total darkness. There are, however, a number of examples of seeds, which germinate better, when they are exposed to light. Kinzel (1966) divided plants into three groups on the basis of influence of light on the germination of seed:

(a) Seeds stimulated to germinate by exposure to light (light sensitive).

(b) Seeds stimulated to germinate by exposure to dark.

(c) Seeds 'indifferent' to illumination.

**Reference**

Kentzer, T. (1966). Gibberellin-like substances and growth inhibitors in relation to the dormancy and after-ripening of ash seeds. *Acta. Soc. Bot.* **35**:575-585.

## Practical No. 14

## Object: To study the method to breaking seed dormancy.

A seed is said to be quiescent, when it does not germinate due to absence of suitable germination conditions. A seed is said to be dormant, when it does not germinate even under the favourable conditions for germination. It is of two types: 1) Exogenous and 2) Endogenous.

**1) Exogenous**

It is a condition, when the entire favourable factors (water, temperature, light) for germination are not available to the seed, hence it fails to germinate. The dormancy is exogenous, when it is due to the presence of an impermeable seed coat to water, gases or solutions. Exogenous is a characteristic of family, Leguminoseae, Chenopodiaceae and Malvaceae family etc.

**2). Endogenous**

The seeds of some plant species do not germinate due to some physiological reasons, which may be due to (a) hormonal imbalance in the embryos, (b) the presence of immature embryo and (c) non-leaching of inhibitors. This type of seed dormancy found in oat, lettuce and in many plant species.

**Methods to Break Exogenous Seed Dormancy**

**A) Scarification**

When seed coat is impermeable to water, gases or offer resistance to expanding embryo. The strength of seed coat is reduced by:

1) **Mechanical Scarification**

This is done to break the dormancy of the seeds, whose seed coat is impermeable to water and gases. It is achieved by rubbing seeds with abrasive surface or with sand or shaking vigorously. Other methods of scarification are: heating, chilling, immersing the seed in organic solvents or piercing the seed coat with needle. These seed treatments alter the seed coat integrity, permitting the penetration of water and gases. Duration of these treatments is critical and it varies from species to species.

**Procedure**

Hard seeds can be screened by imbibing a seed lot in water for 12 hrs. Select the seeds that have not imbibed in water (hard seeds). Place these hard seeds in a mechanical shaker (lined internally with some abrasive surface) for different speeds and for different times. Collect the seed from each speed and time treatment, conduct the germination test. Record

germination (%) after 8 days and determine the optimum speed and time to over come dormancy.

2) **Chemical scarification**

Seeds are treated with chemicals to break dormancy. Sulphuric acid is widely used for this purpose. Organic solvent such as alcohol and acetone have also been used to dissolve the insoluble seed coat constituent and permit the entry of water. Selective hydrolytic enzymes e.g. cellulase, pectinase have also been used to degrade the seed coat. This method can be applied to legumes and Graminae seeds.

**Procedure**

Soak 100 g seeds of Black gram in a beaker overnight. Next day separate the hard seeds from imbibed seeds visually. Treat the hard seeds with concentrated $H_2SO_4$ for 30, 60, and 90 seconds. Wash the seed thoroughly with water, place the seeds for germination, record the observation after 8 days according to standard procedures and observe the difference between various treatments. These are simple and inexpensive methods for removing dormancy. Prolong treatments may damage the seeds, while brief treatments may not break dormancy.

**Methods for Break Endogenous Dormancy**

The endogenous dormancy is due to the inherent properties of the seeds. Unlike exogenous dormancy, which requires a physical alteration of seed coat; it requires physiological changes to break seed dormancy.

Endogenous seed dormancy is of following types:

i) **Rudimentary embryo dormancy:** Seeds of some species are shed before they are morphologically mature. This result in dormancy, because the embryo is immature and unable to germinate. This type of dormancy is found in Ranunculus, Plantago, Fraxinus and Pinus etc. Embryo maturation occurs following dispersal and may take few days to few months.

ii) **Physiological Dormancy:** An equilibrium of endogenous growth promoters and inhibitors regulates seed dormancy in higher plants. The dormancy may be result of presence of inhibitors and absence of promoters. Three hormones have been supposed to play role, in regulating germination. Gibberellins, favours germination, while Cytokinins play a permissive role, whereas, ABA (abscisic acid) inhibits germination. So for germination to occur, the inhibitors (ABA) should be reduced and the promoters ($GA_3$) should be increased.

iii) **Metabolic Inhibitors**: Certain compounds present in the seeds inhibit specific metabolic pathways e.g. cyanide, suppresses the respiration and finally the germination. Phenolic

compounds present in seed coat also inhibit the germination and are regarded as natural inhibitors. Coumarin is main natural germination inhibitor, widely distributed in seeds. It is quickly metabolized during the germination.

**B) Stratification**

Some seeds require exposure to low temperature (3-10$^0$C) for germination followed by transfer to normal temperature required for germination. This process of breaking seed dormancy is called stratification. It is believed that stratification changes the endogenous levels of hormones during exposure to low temperature (i.e. ABA level is reduced and $GA_3$ level is enhanced). The duration of cold temperature may vary from 1 week to 2-3 months, depending upon the type of seed.

**Procedure**

Take two sets of freshly harvested oats seed. Keep one set at 20$^0$C in petri plates lined with filter papers for 10 days, keep other seed set for 5 days at the pre-chilling treatment at 10$^0$C and thereafter subjecting it to normal germination. Observe the differences in germination.

## Practical No. 15

## Object: To study the Seed Viability and Seed Vigour test.

Germination test does not always provide an accurate assessment of plant producing capability of seeds, because in many cases seeds may be viable but fail to germinate due to some reasons. It is essential to check the seed viability and vigour, before the study of effects of allelochemicals, extracts and leachates. Various methods are used to determine the vigour/viability of seeds.

**Seed viability**

It is defined as the degree to which seed is alive, metabolically active and possess all the enzymes capable of catalyzing metabolic reaction needed for germination and seedling growth.

Seeds must be evaluated for quality before planting. The routine test for seed quality i.e., the germination test is a very lengthy process, so this necessitated the search for a quick and effective test to predict seed viability, which helps in timely processing and marketing of seeds. This has become very useful and relevant in the fast expanding seed trade and marketing of costly seeds.

**Method to determine seed viability**

**1. Tetrazolium Test (TZ test)**

**Principle**

The principle of TZ test (Lakon, 1942) is based on the response of all living cells of seeds, which can reduce a colourless salt solution of 2, 3, 5-triphenyl tetrazolium chloride (TTC) or bromide into red coloured compound called formazan. The reduction of TTC takes place in the seeds by a group of dehydrogenase enzymes. Since the reaction takes place within the respiring cells and the formazan is non diffusible, a clear topography of living and non- living tissue is developed.

**Material required**

Blades (preferably) single edged, dissecting needle, petri dish, beaker, watch glasses, magnifying glass, towel paper, filter paper, forceps, dispensing bottle, medicine dropper, tetrazolium salts and weighing balance.

**I. Preparation of Chemical Solutions**

**A) Tetrazolium Solution**

Several concentrations of 2, 3, 5-triphenyl tetrazolium chloride (TTC) or bromide are prepared. The concentrations of the solutions used for intact seeds are 1%, whereas, those

seeds whose embryo is bisected either 0.1 or 0.5% is used. To prepare 1% solution of TZ, dissolve 1g of TZ salt in 100ml of distilled water and then prepare 0.1 and 0.5% concentration by dilulating with distilled water. The pH of solution should be approximately 7.0 and stored in dark or in an amber coloured bottle to prevent photo-deterioration. For best staining, the pH of solution should not vary between 6.0 – 8.0. If pH of water is not in the neutral range, the TZ salts should be dissolve in a phosphate buffer, which is prepared as under.

**Solution 1**

Dissolve 9.078 g of $KH_2PO_4$ in 1000ml distillated $H_2O$.

**Solution 2**

Dissolve 11.876 of $Na_2HPO_4.2H_2O$ in 1000 ml distilled $H_2O$.

Take 400 ml solution 1 and 600 ml solution 2 and mix them. In one liter of buffer solution prepared above dissolve 10g of TZ salt. Thus, the tetrazolium solution of pH 7.0 and 1% concentration is obtained.

**B) Lactophenol solution:**

This solution is used for clearing the grass seeds after staining so that the embryo is visible through lemma and palea. The lactophenol solution is prepared as under:

Mix following in the given ratios: a) 20 parts lactic acid, b) 20 parts phenol, c) 40 parts glycerin, d) 20 parts water

The solution is prepared with medicinal dropper and stored under ambient conditions.

**II. Preparation for seed for testing**

**Conditioning**

Before staining conditioning of seed is required. The seeds that are not readily permeable to water must be made permeable by alternation of seed coat. The moistening of living tissue promotes the activation of enzymes, advancement of embryo activities and improvement of staining.

**Table.3: Type of conditional for different seeds.**

| | Type of conditioning | Nature of seeds |
|---|---|---|
| a) | No moistening or additional preparation | Small seeded legumes with permeable seed coat |
| b) | Removal of seed coat | Dicot seeds |
| c) | Piercing, puncturing or cutting of seed coats | Small seeded crops |
| d) | Longitudinal cut through mid section of the embryo and endosperm | Monocots |

**Procedure**

After conditioning wheat seed in water for 8 hrs, give a longitudinal cut through the mid section of the embryo and endosperm. Discard one part of the cut seed and put the other half in 0.1% TZ solution. Keep the petri plate in a dark place for 2 hrs at room temperature (20 $^0$C to 40 $^0$C). Higher temperature (>45$^0$C) is not recommended. When short staining tests are desired, then place the test sample in germinator at 30-35$^0$C. Generally the staining is done twice as fast, when the temperature increases by 10 $^0$C in the range of 20-40$^0$C.

**Staining**

After staining is complete, interpret the results. The seed viability index of monocot and dicot seeds are evaluated based on following observations:

**Monocot seeds staining:** various organs may stain as:

a) Growing tips of the embryonic axis especially plumule.

b) Point of attachment of the embryo to scutellum.

c) Region of seminal root emergence.

**Dicot seed staining:** various organs may stain :

a) Radicle and hypocotyls development.

b) Cotyledons

c) Plumule region

Proper staining of above seedling parts indicates that seeds are viable, while improper staining in these areas indicate non-germinable or abnormal seeds.

**Advantages**

i) It is a quick estimate of viability.

ii) Viability (TZ) test is extremely useful in dormant or very slow germinating seeds.

iii) Seeds are not damaged (only in dicots) and same seeds can be used for conducting germination test.

**Disadvantages**

1) It is difficult to distinguish between the normal and abnormal seedlings and between dormant and non-dormant seeds.

2) As TZ test does not involve germination, the microorganisms harmful to the germinating seedling are not detected.

3) The knowledge of seed and seedling structure is essential.

**Precautions**

1) Seed sample should be completely covered with the TZ solution.

2) The staining should be done in dark.

3) The prolonged exposure to strong light oxidizes the testing solution and results in abnormal tissue colour.

4) When seeds are not to be evaluated immediately after staining, replace the TZ solution with water.

5) To evaluate the small seeded grasses, apply lactophenol clearing solution after staining.

6) Remove the seed coat of legumes with the forceps and teasing needles before examination.

7) Lactophenol is corrosive, therefore, avoid skin contact or inhalation.

**Seed vigor**

Seed vigor is defined as those properties of seed, which determine the potential for rapid and uniform emergence and development of normal seedling under wide ranges of conditions (McDonalds, 1980).

**Seed vigor test**

The characteristic of seed vigor test are: (i) It should be easy and rapid to perform, (ii) It should be reproducible, (iii) It should be economical, (iv) It should correlate with field emergence.

**Colorimetric vigour test (Kittock and Law, 1968)**

The reduction of 2, 3, 5-triphenyl tetrazolium chloride (TTC) to red colour formazan by dehydrogenase enzymes in seed embryo is the basis of topographical test of seed viability. We describe the quantitative method, which may be used to determine the varying dehydrogenase activity between seed lots of similar viability and hence it is used as measure of vigour.

**Material required**

Spectrophotometer, centrifuge, balance, grinding mill, cuvette, centrifuge tubes, TZ solution, acetone and oven.

**Procedure**

1. Grind 1 g seed in mill with No. 20 screen sieve.
2. Weigh out 200 mg ground seed and put it in centrifuge tube.
3. Add 1.5-5.0 ml of 1% TTC solution (2,3,5-Triphenyl tetrazolium chloride)of pH 7.0 and shake.
4. Incubate the mixture for 2 hours at 35°C
5. Centrifuge the mixture for 3 minutes at 10,000 rpm. Pour off TTC solution.
6. Add 5 to 10 ml acetone to the test tube to extract formazan.

7. Keep the centrifuge tube as such at room temperature for 16 hour.

8. Centrifuge for 3 minutes at 10,000 rpm.

9. Decant acetone solution and read absorbance (OD) at 480 nm/520 nm.

**Interpretation**

Higher OD will indicate more viability of seeds.

**Precautions**

Incubation temperature should be maintained at 35± 1°C.

**References**

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## Practical No. 16

## Object: Estimation of Phenols.

Phenols constitute a class of aromatic organic compounds having at least one hydroxyl group attached directly to the benzene ring.

They are also called as carbolic acid and phenic acid. Plant phenols include gossypol, tyrosine, tannins and flavonols etc. phenols are found in almost all parts of a plant; however, they are more concentrated in fruits (such as grapes), raisins, garlic, onion and green tea. Phenols are considered to offer resistance to pests and diseases in plants. Phenols have medicinal value also. They may protect humans against some cardiovascular diseases. In plants, phenols are found free as well as bound to cell wall. However, the amount of cell wall phenol is significantly lower than that of the free phenol.

Total phenols is determined following Folin-Ciocalteau assay (Campbell and Ellis, 1992; Julkunen-Tiitto, 1985; and Funk and Brodelius, 1990).

Take 0.5 to 1.0 g of sample, grind it in liquid nitrogen using mortar and pestle.

↓

Scoop the power into a 5 ml polystyrene tube with cap (sample may be replicated four times).

↓

Extract it with 2 – time volume of 50% methanol for 2 hour at 80° C in a water bath.

↓

Centrifuge the mixture for 10 minutes at 3000 rpm and use the supernatant for Folin - Ciocalteau assay.

↓

Take 50 µl of supernatant, dilute it to 1 ml with distilled water and then mix with 0.5 ml of Folin-Ciocalteau reagent (M/s Sigma) and 2.5 ml of 20% of $Na_2 CO_3$

↓

Allow the mixture to stand for 20 minutes at room temperature and then measure the absorbance at 725 nm wavelength using a spectrophotometer.

↓

The phenol concentration is determined from a standard curve prepared from p-coumaric acid (M/s Sigma).

↓

It may be mentioned that this method estimates the free phenols present in the sample.

For extraction of ester-bound cell wall phenols, the following steps should be followed:

After centrifugation, use the pellets (not the supernatant) for extraction of cell wall phenols.

Saponify the pellets with 0.5 M-NaOH in a ratio of 1:4 for 24 hrs at room temperature.

Neutralize the mixture with one-forth volume of 2 M-HCL.

Centrifuge for 5 minutes at 3000 rpm and use the supernatant for Folin-Ciocalteau assay (as described above).

**References**

Campbell, M.M. and Ellis, B.E. (1992). Fungal elicitor-mediated responses in pine cell cultures. *Planta* 186: 409-417

Funk, C. and Brodelius, P.E. (1990). Influence of growth regulators and an elicitor on phenyl propanoid metabolism in suspension cultures of *Vanilla planifolia*. *Phytochem* 29: 845-848.

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## Practical No. 17

## Object: Estimation of Reducing, Non-Reducing and Total Sugars.

The most abundantly and commonly available compounds in the plant kingdom are carbohydrates. They comprise saccharides viz.

Monosaccharides – Glucose, Fructose etc.

Disaccharides – Sucrose, Lactose, Maltose etc.

Polysaccharides – Starch, Cellulose etc.

Cellulose is the major component of plant cell wall.

The active groups in carbohydrates are aldehyde (-CHO) and ketone (=CO). In addition, they contain hydroxyl groups as well. The chemical properties of saccharides vary depending upon the number of hydroxyl groups and the presence or absence of (-CHO) or (=CO) groups.

### I. Estimation of Reducing Sugars

**Principle**

The reducing property of sugars arise out of the presence of a potential aldehyde or keto group. Nelson (1944) and Somogyi (1952) method is the most common and widely used method for quantitative determination of reducing sugars. In this method, alkaline copper tartrate and arsenomolybdic acid are used as the major reagents. Reducing sugars reduce copper from cupric ($Cu^{2+}$) to cuprous state ($Cu^{+}$) which in turn, reduces molybdic acid into molybdenum, resulting into development of blue colour. OD is measured at 620 nm in a colorimeter.

**Preparation of Reagents**

***(a) Arsenomolybdate colour reagent***

Dissolve 25 grams of ammonium molybdate [$(NH_4)_6\ Mo_7O_{24}\ 4H_2O$] in 450 ml of distilled water. Add 21 ml of concentrated $H_2SO_4$ to it and mix properly. To the above solution, an aqueous solution of sodium arsenate (3g/25ml) is added and mixed. The final solution is placed is an incubator at $37^0$ C for 48 hrs.

***(b) Alkaline-copper-carbonate-tartrate reagent (Somogyi reagent)***

- Dissolve 24 grams of anhydrous sodium carbonate and 12 gram of Rochelle salt (Na-K tartrate) in 250 ml of water. Add 40 ml of 10% copper sulphate solution into the above solution with stirring, followed by addition of 16 gm of sodium bicarbonate (Solution A).
- Dissolve 18 grams of sodium sulphate in 500 ml of hot water and boil to expel air (Solution B).

- On cooling, mix solutions A and B and make final volume upto 1000 ml with distilled water.

***(c) Standard glucose solution***

*Stock:* Dissolve 100 mg sucrose in distilled water and make the volume upto 100 ml. Take 10 ml of this stock solution and dilute it to 100 ml distilled water (100 µg/ml). This solution serve as the working standard.

**Estimation of Sugars**

***(i) Sample preparation***

Fresh leaf sample (1gm) is collected on clear day and preserved in 95% ethanol in glass vials.

***(ii) Extraction of sugars***

For sugar analysis, supernatant (alcohol in which the leaf material is plunged) is decanted into a beaker. The extraction is repeated 3 to 4 times by boiling the sample with 50 to 20 ml of 80% (v/v) ethanol in water each time and decanting the supernatant in the same beaker (Mc Cready *et al*, 1950). The combined sugar extract is made upto a final volume of 100 ml with distilled water in a volumetric flask.

***(iii) Purification of sugar extract (removal of colloidal particles)***

50 ml aliquot of the above sugar extract is evaporated in a water bath taking care not to let the liquid dry out completely. The sample is then treated with one ml saturated solution of lead acetate (to precipitate colloidal substances) and then filtered into a beaker containing 3.0 ml of saturated disodium hydrogen phosphate. Lead is precipitated as lead phosphate. After 2-3 washings the content of beaker is filtered into a 50 ml volumetric flask and made upto a final volume of 50 ml. An aliquot of this solution is used for determining the reducing sugars by Nelson's arsenomolybdate method (Nelson, 1944). Improved copper reagent of Somogyi (1952) is used for this purpose.

***(iv) Determination of reducing sugars***

a). Take 0.2 ml of the aliquot in a test tube and make the volume upto 2 ml with distilled water.

b). Add 1 ml of Somogyi copper reagent and heat in a boiling water bath of 10 minutes.

c). After cooling the sample in running tap water, 1 ml of arsenomolybdate reagent is added and final volume made upto 10 ml with distilled water. OD is measured at 530 nm in a spectrophotometer or colorimeter.

d). From the graph drawn (using working standard solutions) calculate the amount of reducing sugars present in the sample.

**Calculation**

Absorbance corresponds to 0.1 ml of the test solution = x mg of glucose.

100 ml contains $\frac{X}{0.1}$ x100mg of glucose.

= % of reducing sugars.

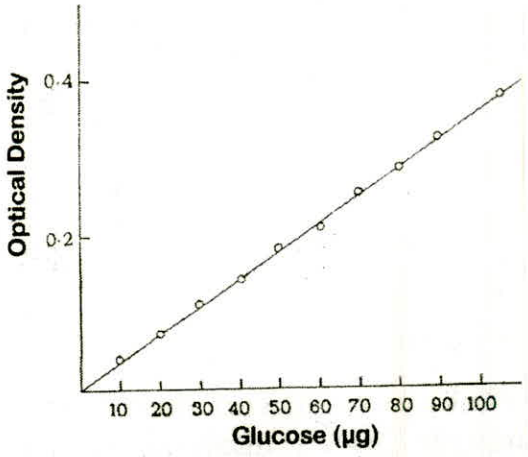


**Fig.14. Standard curve for glucose**

## II. Non-Reducing Sugars

Non- reducing sugars are calculated by subtracting the reducing sugars from the total sugar content.

## III. Estimation of Total Sugars

5 ml of sugars extract (prepared as above) is hydrolysed by boiling with 2.5 ml of 0.5 N-HCl for 30 minutes in a water bath and later neutralized to slightly acidic side with 0.5 N-NaOH. The final volume is made upto 10 ml with distilled water and the solution is used for determining total sugars. An aliquot of above solution is analysed for total sugars as described in **step c**. OD is measured at 530 nm.

**References**

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Somogyi, M. (1952).Notes on sugar determination. *J. Bio. Chem.* 195: 19-23.

## Practical No. 18

## Object: Estimation of Total Carbohydrate.

Total carbohydrate in a plant sample is estimated by phenol sulphuric acid method. It is a simple, easy and convenient method. The carbohydrate is analysed quantitatively with the help of a colorimeter. OD is recorded at 490 nm.

**Reagents required**

(i) *Phenol solution (5%):* 50 gm of phenol (reagent grade) is dissolved in water and the volume is made up to one litre.

(ii) *Sulphuric acid:* 96% (Analytical grade)

**Method of Extraction**

Method of extraction of total carbohydrates is the same as in case of starch extraction.

0.5 g of the homogenized in 80% hot ethanol (vol. 10-20). The slurry is centrifuged at 10,000 rpm for 10 minutes. The supernatant is discarded and residue is retained. The residue is washed 2-3 times with 80% ethanol and finally it is dried over a hot water bath.

To the residue, 5 ml of water and then 6.5 ml of 52% perchloric acid is added. It is extracted at $0^0$ C for 20 minutes. Then it is centrifuged and the supernatant is preserved. Extraction with fresh perchloric acid (52%) and centrifugation are repeated 2-3 times. The supernatant is collected and the final volume is made up to 100 ml with distilled water. Similarly standard glucose solution and working standards should also be prepared.

**Procedure for Carbohydrate Assay**

Take 0.1 and 0.2 ml of the sample extract in two separate test tubes and make up the volume in each test tube to 1 ml with distilled water. Set a blank with 1 ml of water. Add 1 ml of phenol solution and thereafter 5 ml of 96% sulphuric acid to each test tube and shake well. After 10 minutes, incubate the samples in a water bath at $30^0$ C for 20 minutes and allow the sample tubes to the room temperature. Measure OD at 490 nm of wavelength using a colorimeter.

**Calculation**

Use a standard curve of glucose solutions, to get the absorbency of 0.1 ml of the test solution with 'x' mg of glucose.

Hence, 100 ml of the sample will contain. $\frac{x}{0.1}$ x100 mg of glucose

= % of total carbohydrate present

# Appendix

**Table. 1: Conversion units of wavelength**

| | |
|---|---|
| 1 micron | = 1μ<br>= 1 μm<br>= $1x10^{-6}$m<br>= $1x10^{3}$ nm<br>= $1x10^{4}A^{0}$ |
| 1 $A^{0}$ | = 0.1nm<br>= $1x10^{-4}$ μm<br>= $1x10^{-10}$ m |
| 1 nm | = $10A^{0}$<br>= $1x10^{-3}$ μm<br>= $1x10^{-9}$ m |

**Table. 2: Visible spectrum with the wavelength rang (nm)**

| | |
|---|---|
| Ultra violet | <400 nm |
| Violet | 400-425 nm |
| Blue | 425-490 nm |
| Green | 490-560 nm |
| Yellow | 560-580 nm |
| Orange | 580-640 nm |
| Red | 640-740 nm |
| Infra Red (IR) | >740 nm |

**Table. 3: Distribution of stomata and their pore size in the leaves of common plants**

| Plants | Stomata $cm^{-2}$ | Aperture* (diameter in μm) |
|---|---|---|
| Bean | 28100 | 5.4 |
| *Begonia* | 4000 | 15.6 |
| Castor bean | 17600 | 7.6 |
| *Coleus* | 14100 | 7.9 |
| English ivy | 15800 | 8.3 |
| *Geranium* | 5900 | 15.9 |
| Maize | 6800 | 13.9 |
| Oat | 2300 | 27.5 |
| Sunflower | 15600 | 16.5 |
| Tomato | 13000 | 10.4 |
| Wheat | 1400 | 27.4 |
| **Average** | **11327** | **14.2** |

*Aperture is calculated from length x width and assumes a perfect circle. (Data from Verduin, 1949)

**Table. 4: The water potential of sucrose solutions at $2^0$ C in bars**

| Molarity (M) | Water Potential (ψ) | Molarity (M) | Water Potential (ψ) |
|---|---|---|---|
| 0.0 | 0.00 | 1.0 | -34.6 |
| 0.1 | -2.64 | 1.1 | -39.8 |
| 0.2 | -5.29 | 1.2 | -45.4 |
| 0.3 | -8.13 | 1.3 | -51.6 |
| 0.4 | -11.11 | 1.4 | -58.4 |
| 0.5 | -14.31 | 1.5 | -65.8 |
| 0.6 | -17.77 | 1.6 | -73.9 |
| 0.7 | -21.49 | 1.7 | -83.0 |
| 0.8 | -25.54 | 1.8 | -93.2 |
| 0.9 | -29.70 | 1.9 | -104.5 |

(Data from Walter, 1931)

**Table. 5: Climatric and non-climatric fruits**

| Climatric | Non-Climatric |
|---|---|
| Apple | Bell pepper |
| Avocado | Cherry |
| Banana | Citrus |
| Fig | Grape |
| Mango | Pineapple |
| Olive | Strawberry |
| Peach | Watermelon |
| Pear | |
| Persimmon | |
| Plum | |
| Tomato | |

(*Source:* Kadar and Barrette, 2003)

**References**

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## PREFACE

Crop Physiology is the basic science which caters to the need of several cognate disciplines. The present practical manual entitled 'Practical Manual on Crop Physiology' has been prepared for the students of B.Sc. (Ag) and M.Sc. (Ag) programme as per the syllabus approved by 4th Dean's committee.

To Elucidate a physiological process, one has to undertake quantative and qualitative analysis of a specific parameter. Various important physiological processes like seed germination, transpiration, plant growth and development, photosynthesis, respiration, imbibition, seed viability and vigour etc. are included in this manual. I have tried to provide appropriate solutions to the physiological problem, even with or without the most sophisticated laboratory instruments and equipments. The users of this practical manual can select suitable methods, according to the available facilities. I have made sincere efforts in presenting the procedure of various methods in simple way. All efforts have been made to provide the references also so that students can refer book and original articles for more information.

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All necessary precautions have taken to prepare the text free of mistakes but if you come across with any of such mistakes; it would be cordially invited to improve it in future.

**Place: Kawardha:**
**Dated: September 12, 2012**

**(Vidya Bhushan Kuruwanshi)**
**Author**

छत्तीसगढ़ संवाद

# PRACTICAL MANUAL ON CROP PHYSIOLOGY

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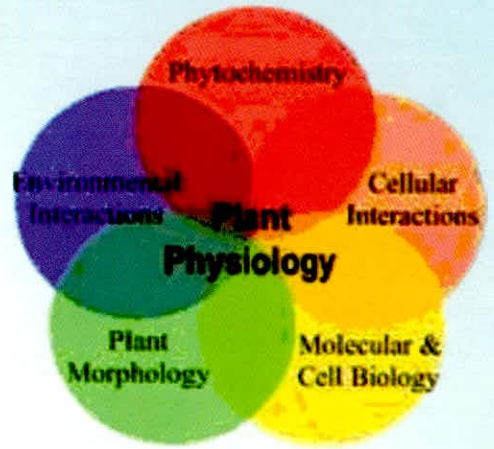
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