C.O.H./PRACTICAL MANUAL- 3 / 2013

Practical Manual on Growth and Development of Horticultural Crops

(As per recommendations of 4th dean's committee)

B.Sc. (Horticulture)
(2013)

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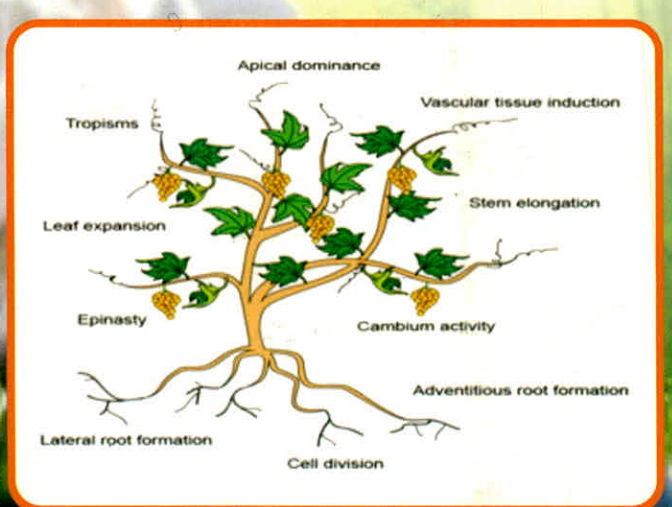

COLLEGE OF HORTICULTURE, RAJNANDGAON
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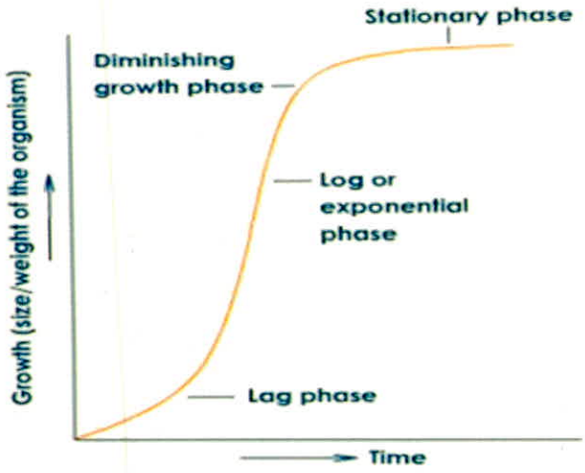
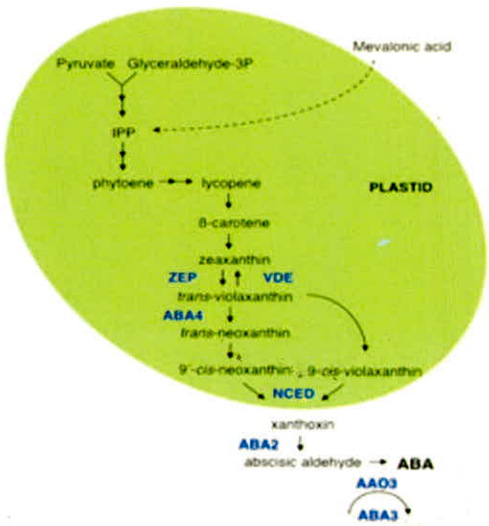
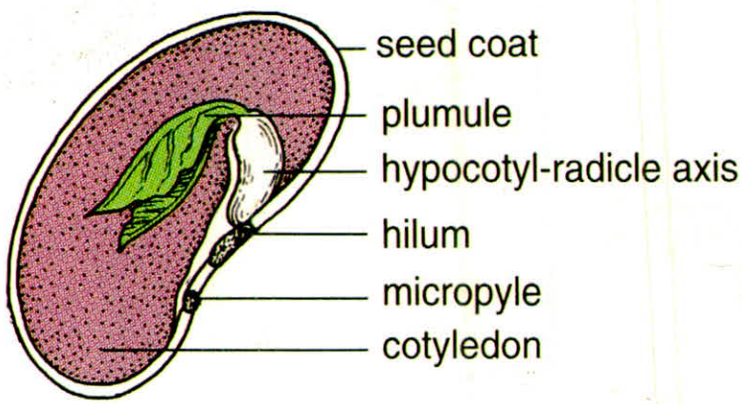
Edition: 2013 (First)

Published by
Dean
College of Horticulture,
Indira Gandhi Krishi Vishwavidyalaya
Rajnandgaon - 431 445







PHYSIOLOGICAL DISORDERS IN DIFFERENT FRUIT CROPS

Chilling injury in banana

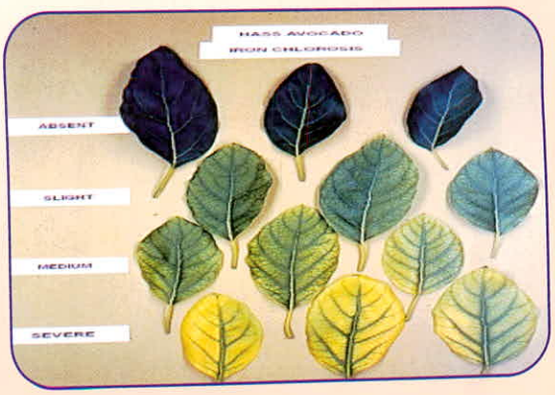
K deficiency in banana

Maturity Bronzing

N deficiency in banana

Zn deficiency in citrus

Zn deficiency in banana



Fe chlorosis in Acid lime

Hen and chicken disorder of grape

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INDEX

Exercise 1: Plant growth and development mechanism.

What is plant growth?
Plant Growth can be defined as

"Vital process which brings about a permanent change in any plant or its part in respect to its size ,form,weight and volume."

OR

"An irreversible permanent increase in size of an organ or its parts or even of an individual cell."

It may be evaluated by measurements of mass, length or height, surface area or volume. Generally, growth is accompanied by metabolic processes (both anabolic and catabolic), that occur at the expense of energy. Therefore, for example, expansion of a leaf is growth.

What is Development?

Development is a term that includes all changes that an organism goes through during its life cycle from germination of the seed to senescence. Diagrammatic representation of the sequence of processes which constitute the development of a cell of a higher plant is given in Figure1.1.1

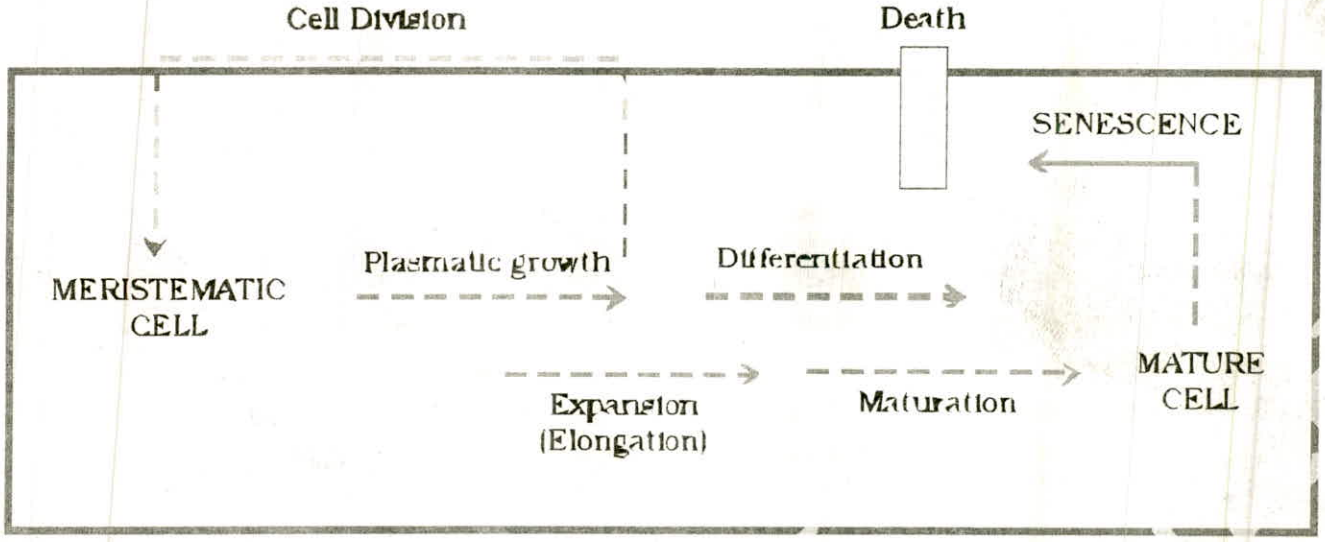


Figure1.1 : Sequence of the developmental process in a plant cell.

Development is the sum of two processes: growth and differentiation.To begin with, it is essential and sufficient to know that the development of a mature plant from a zygote (fertilised egg) follow a precise and highly ordered succession of events. During this process a complex body organisation is formed that produces roots, leaves, branches, flowers, fruits, and seeds, and eventually they die (Figure 1.2).

Development = growth + differentiation

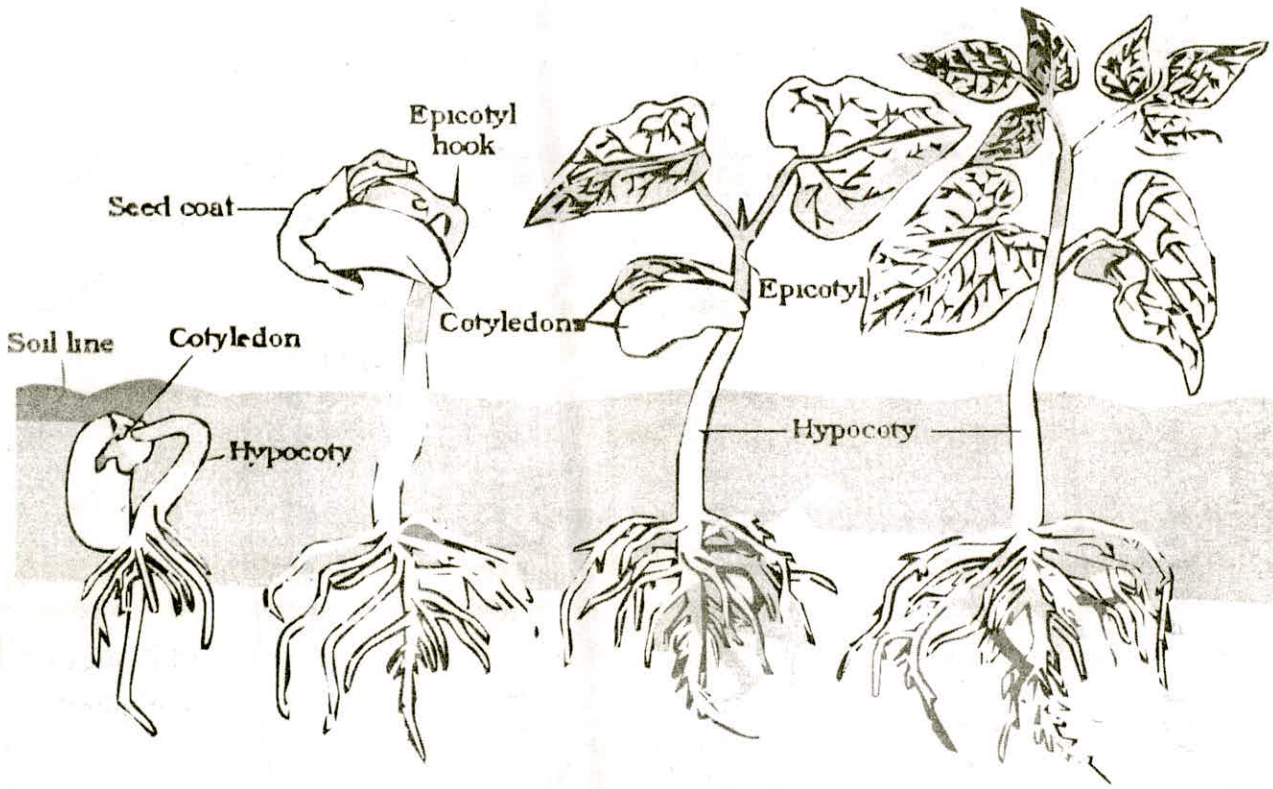


Figure 1.2 : Germination , growth and seedling development

How does a plant growth and develop their stages ?

Growth is regarded as one of the most fundamental and conspicuous characteristics of a living being. Plant growth is unique because plants retain the capacity for unlimited growth throughout their life. This ability of the plants is due to the presence of meristems at certain locations in their body. The cells of such meristems have the capacity to divide and self-perpetuate. The product, however, soon loses the capacity to divide and such cells make up the plant body. This form of growth wherein new cells are always being added to the plant body by the activity of the meristem is called the open form of growth (Figure1. 3).

Growth Stages

1. Embryogenesis [part of seed development]
   Zygote ⟶ Embryo

2. **Vegetative development includes**

(a). Seed germination

From a heterotrophic to a photosynthetically-competent seedling

(b). Development of the Vegetative plant

Indeterminate growth regulated by environmental factors

3. **Reproductive development**

Flowering ◂ pollination ◂ fertilization ◂ zygote

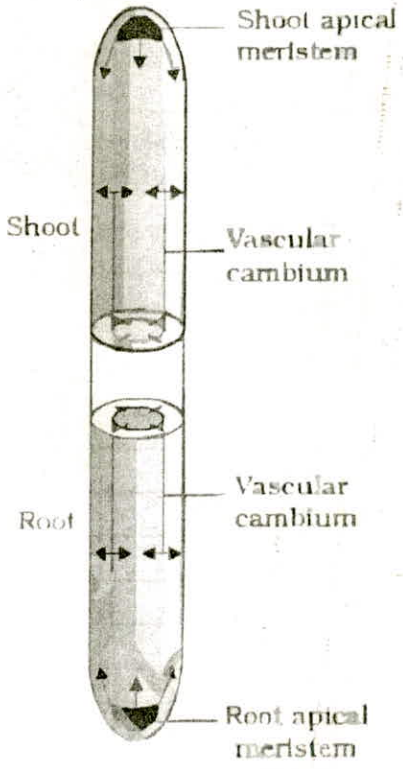


Figure 1.3. Diagrammatic representation of locations of root apical meristem, shoot aplical meristem and vascular cambium. (Arrows exhibit the direction of growth of cells and organ)

What are the principles of plant development?

1. Expression of genes that encode transcription factors determine cell, tissue, and organ identity.
2. Fate of cell is determined by its position, not its clonal history.
3. Development pathways are controlled by networks of interacting genes
4. Development is regulated by cell-to-cell signaling. Ligand- small proteins, CHO, Hormone signaling

Patterns of Growth and Development

Growth in plants is restricted to certain zones, recently produced by cell divisionin a **meristem.** It is easy to confuse growth (as defined above as an increase in size) withcell division in meristems. Cell division alone does not cause increased size, but thecellular products of division also increase in volume and cause growth. Root and shoot tips **(apices)** are meristematic in nature. Other meristematic tissues are found in the vascular cambium and just above thenodes of monocots or at the bases of grass leaves(Fig 1.4) .

(A) The root and shoot apical meristemsare formed during embryo development, while the seed develops and are called **primary meristems**.

(B) The vascular cambium and the meristmatic zones of monocot nodes andgrass leaves are indistinguishable until after germination; they are **secondary meristems**.

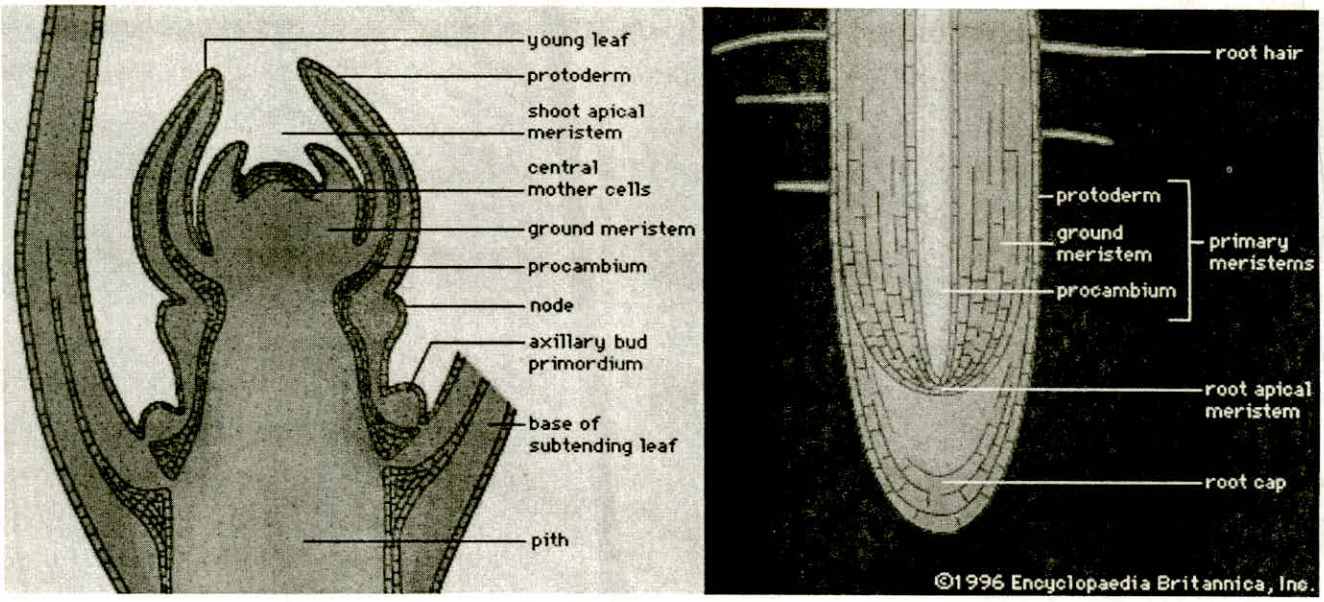
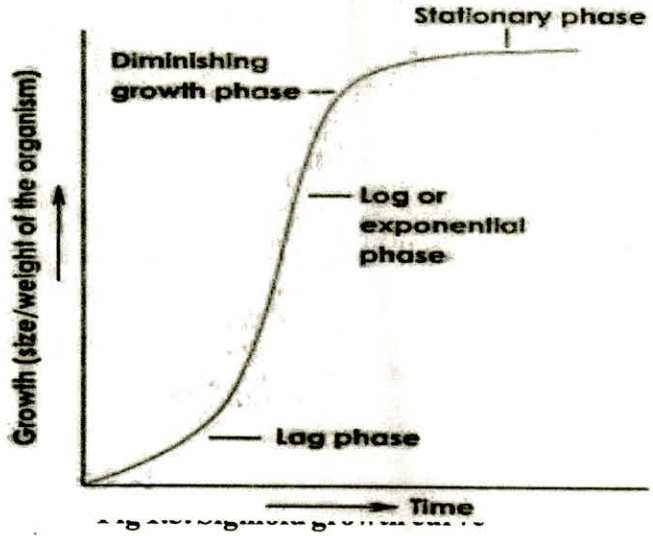


Fig 1.4 (A) Shoot system with apical meristem **(B) Root system with apical meristem**

Kinetics of growth (Growth curve)

Under favorable conditions there is a characteristic increase in the plant's growing parts. Growth is slow at first (Lag Phase), then gains speed (Log Phase) and eventually slows down (decreasing growth Rate) to come to a halt (Steady state). The total time during which this course of growth takes place is called as the grand periodof Growth. If this growth rate is plotted against time, a slanting S shaped curve isobtained which is called as Sigmoid Curve or Grand Period Curve(Fig1.5).The sigmoid curve represents the integrated sum of the curves for each growingorgan and cell and presents the changing size of these parts.Similarly, when dry weightis measured as an index of growth before maturity, the curve takes the well knownsigmoid form. Environmental conditions may alter growth rates but not the sigmoid form of the growth curve.



GROWTH RATES

The increased growth per unit time is termed as growth rate. Thus, rate of growth can be expressed mathematically. An organism or a part of the organism can produce more cells in a variety of ways.The growth rate shows an increase that may be arithmetic or geometrical (Figure.4). In arithmetic growth, following mitotic cell division, only one daughter cell continues to divide while the other differentiates and matures. Mathematically, it is expressed as

$\boxed{L_t = L_0 + rt}$

L_t = length at time 't'

L_0 = length at time 'zero'

r = growth rate / elongation per unit time.

In geometrical growth, the initial growth is slow (lag phase), and it increases rapidly thereafter - at an exponential rate (log or exponential phase). Here, both the progeny cells following mitotic cell division retain the ability to divide and continue to do so. However, with limited nutrient supply, the growth slows down leading to a stationary phase.

Blackman (1919), an English plant physiologist, suggested that the exponential growth rate of the plant could be represented by an equation

$W_1 = W_0 e^{rt}$

W_1 = final size (weight, height, number etc.)

W_0 = initial size at the beginning of the period

r = growth rate

t = time of growth

e = base of natural logarithms

r = relative growth rate

The above equation also indicates that the size of an organism **(W1)** depends on the initial size **(Wo)**. The plant size also depends on the magnitude of r (relative growthrate). It should be noted that ***'r'*** is the relative growth rate. Blackman suggested that ***'r'*** might be used as a measure of the ability of a plant to produce new plant material andcalled ***'r'*, the efficiency index**. The plants with high efficiency index could be expected to outperform plants with a low efficiency index. Although ***'r'*** does differ among plant species, it is not constant during the life of a plant.

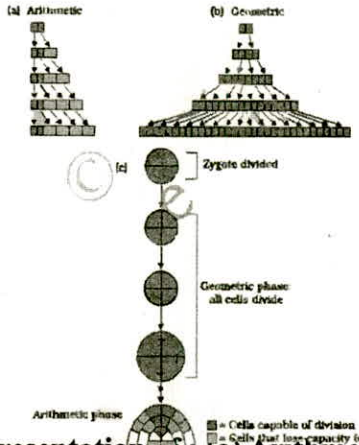
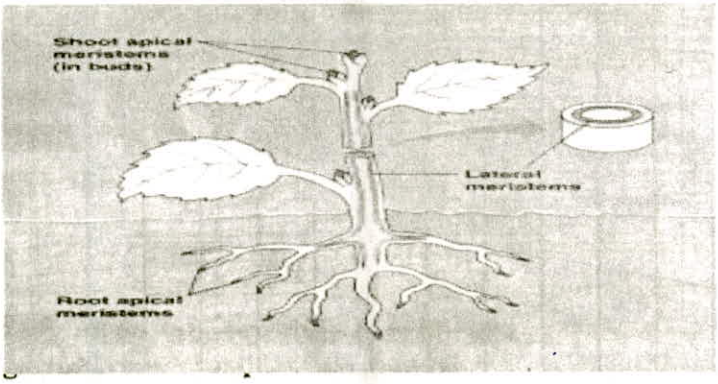


Figure 1.6. Diagrammatic representation of : (a) Arithmetic (b) Geometric growth

Types of Growth (Based on Developmental Stages)

Primary growth: Apical meristems extend roots and shoots by giving rise to the primary plant body.
Secondary growth: Lateral meristems add girth by producing secondary vascular tissue and periderm.



Factors affecting plant

1) Internal factors

a. Resistance to biotic and abiotic stresses
b. The rate at which plants show resistance towards biotic and abiotic stresses
c. Respiration
d. Partitioning of assimilate and nitrogen
e. Capacity to store food resources
f. Enzyme activity
g. Direct gene effects (eg. Heterosis).

2) Environmental factors

A) Climatic

i. Light
ii. Temperature
iii. Water
iv. Photoperiod
v. Gases

B) Edaphic (soil factors)

i. Texture
ii. Structure
iii. Organic matter
iv. CEC
v. pH and
vi. Nutrient availability

C) Biological

i. Weeds
ii. Insects
iii. Diseases
iv. Nematodes, and
v. Soil microorganism

EXCESISE 2: Growth and development analysis parameters used in horticultural crops.

What is plant growth analysis?

The term plant growth analysis refers to a useful set of quantitative methods that describe and interpret the performance of whole plant systems grown under natural, semi natural or controlled conditions. Plant growth analysis provides an explanatory, holistic and integrative approach to interpreting plant from and function. It uses simple primary data such as weights, area, volumes and contents of plant or plant component to investigate process with in involving the whole plant or crops.

The analysis of yield influencing factors and plant development as net photosynthate accumulation integrated over time is also known as growth analysis.

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.

So it is a useful tool in studying the complex interactions between plant growth and the environment. Growth analysis measurements do not require elaborate equipments or extensive laboratory facilities, but need just an oven and a balance for dry weight determination and a method for measuring leaf surface. The method employed for measuring the growth may also provide better understanding of growth processes and limitations of crop yield.

PARAMETERS OF GROWTH ANALYSIS

1) Leaf Area (LA)

It is the surface area of leaf, which aids in photosynthesis. Total leaf area per plant is often a useful measurement in bio-productivity studies. Area can be measured by graphic methods, weight method, by using Leaf Area Meter and by measuring length and breadth of the leaf.

A) Weight method

$$LA = \frac{X}{A} \times B$$

Where,

X = known area of leaves, A = dry weight of known area of leaf

B = dry weight of unknown area of all leaves.

The leaf area of standard leaves, usually 3rd leaf from the top is measured and dried.

(B) Linear measurement method

$$LA = LBK\ (cm^2 \text{ or } m^2)$$

Where,

L= maximum length,

B= maximum breadth,

K= constant (can be worked out by regression analysis)

K value may not be the same for different varieties in the same species.

(2) Leaf Area Index (LAI)

Watson (1947) proposed the term leaf area index which is the ratio of the leaf area of a plant to the ground area occupied by the plant.

$$\text{Leaf area index} = \frac{\text{Total leaf area of the plant}}{\text{Ground area occupied by the plant (spacing)}}$$

3) 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 expressed in terms of cm2g-1.

$$LAR = \frac{\text{Leaf area per plant}}{\text{Total plant dry weight}}$$

4) 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. It also measures the persistence of the assimilating surface. This factor was suggested by Power et al. (1967) and expressed in days.

$$LAD = \frac{L(i) + L(ii)}{2} \times (t2 - t1)$$

LAI (i) - Leaf area index at first stage, LAI (ii) - Leaf area index at second stage

t2 -t1 - Time interval between the two consequent stages and expressed in days.

5) Leaf Area Ratio (LAR)

In order to estimate the carbon assimilatory efficiency of leaves or to estimate the leafiness of plants, Radford (1967) suggested leaf area ratio as a measure of leaf area to the weight of the whole plant. It is expressed as cm^2 g-1. In broad sense, LAR represents the ratio of photosynthesizing to respiratory material within the plant.

$$LAR = \frac{\text{Leaf Area/ plant}}{\text{Plant dry weight}}$$

(6) Specific Leaf Area (SLA)

It is the ratio of assimilating area to its dry weight. Following formula was proposed by Kvet ***et al.*** (1971) to arrive SLA and expressed as cm^2 g-1.

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

(7) Specific Leaf Weight (SLW)

Using the leaf dry weight and leaf area, SLW is calculated. It is the ratio of leaf dry weight to its area of assimilating surface. The formula was suggested by Pearce et.al (1968) and expressed as mg cm-2.

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

(8) Leaf Weight Ratio (LWR)

It is the ratio of total leaf dry weight to the whole plant dry weight. It is the measure of leafiness of the plant on a weight basis. It is expressed in g kg-1.

$$LWR = \frac{\text{Leaf dry weight}}{\text{Total plant dry weight}}$$

9) Net Assimilation Rate (NAR)

It is the rate of increase of leaf by dry weight per unit area of leaf per unit time.Williams (1946) employed the formula and expressed as mg cm^{-2} day -1

$$NAR = \frac{\text{loge L2 - Loge L1}}{\text{L2 - L1}} \times \frac{\text{W2 - W1}}{\text{t2-t1}}$$

Where,

Log e L2 = Natural log of leaf area at stage 2.

Log e L1 = Natural log of leaf area at stage 1.

L2 & L1 = Leaf area at stage 2 & 1 respectively

W2 & W1 = Dry weight of the whole plant at stage 2 & 1 respectively

t2-t1 = Time interval between the two stages.

(10) Relative Growth Rate (RGR)

It is the rate of increase of dry weight per unit weight already present per unit time. Williams (1946) suggested the formula and expressed as g g-1 day-1

$$RGR = \frac{\text{Loge W2 - loge W1}}{\text{t2 - t1}}$$

Where,

W2 & W1 = Whole plant dry weight at t2 & t1 respectively.

(11) Crop Growth Rate (CGR)

CGR is a simple and important aid of agriculture productivity. It is the rate of increase of dry weight per unit land area per unit time. Watson (1958) suggested the formula and expressed as g m^{-2} day-1

$$CGR = \frac{W2 - W1}{P(t2 - t1)}$$

Where,

W2 and W1 = are plant dry weight at time intervals t2 and t1

P = population per unit area

CGR is also the product of leaf area index and net assimilation rate.

$$CGR = LAI \times NAR$$

(12) Harvest Index (HI)

It reflects the proportion of assimilate distribution between economic yield and total biomass yield (Donald and Hamblin, 1976).

$$HI = \frac{\text{Economic yield}}{\text{Total biomass yield}}$$

EXCESISE 3: Measurement of leaf area by different methods.

What is leaf area?

Leaf area means total area/surface of leaf which is used in production of biomass or photosynthesis. Generally area of leaf is expressed in cm^2.

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 anessential 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. Following are the most simple, inexpensive and accurate methods:

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

1. Graphic method

This involves the use of graph papers for the estimation of only smaller leaves. Sothis type of method cannot be used for estimating the leaf area for all types of leaves. Forestimating 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 (Figure 3.1). The leaf area isexpressed as cm^2 per leaf.

Figure 3.1. Diagrammatic representation of Graphic method

2. Electronic method (leaf area meter)

Leaf Area Meter is used for estimating leaf area of all types of leaves. This methodis also termed as direct method. But the leaves should be removed or detached from theplants 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 conveyorbelt are provided. Initially zero is set. When a leaf is placed in the conveyor belt it movesalong with conveyor belt and when the leaf comes close to scanning camera, it reflectsthe image of leaf on the mirror and the reading is measured digitally (Figure 3.2). Area of leaf isexpressed in cm^2.

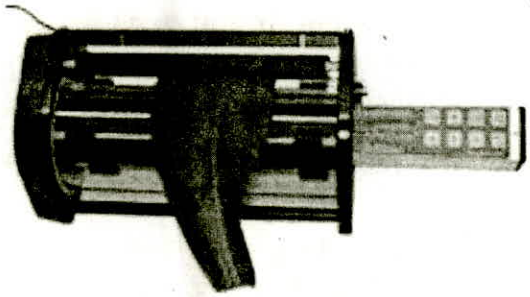


Figure 3.2 : leaf area meter.

3. Linear Method

This method is relatively simple, time saving and non-destructive method for estimating the leaf area. 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

The value of leaf area constant (K) is the ratio between actual leaf area and apparent leaf area and is always less than 1. Apparent leaf area can be calculated by multiplying the maximum length and breadth of the leaf. Actual leaf area can be measured by using Leaf Area Meter. The Leaf area constant may not be same for different varieties and different growth stage also.

Approximate K value for important crops

Crops	Equation
Banana	A=0.756
Grapes	A= 0.81
Papaya	A=106 X -2028 X= length of median midrib (mm)
Tomato	A=0.41 +0.211 X X= L x W
Bhendi	A=115 X -1050 X= length of midrib (mm)
Onion	A=2.794 + 1.686 X X = leaf length
Acid lime	A= 0.608

4. Weight basis method

The leaf area (L1) occupied by known dry weight (Wl) 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.

$$\text{Whole leaf area (L2)} = \frac{L1}{W1} \times W2$$

Where,

L2 - Total leaf area, L1 - Single leaf area,

Wl - Single leaf weight (dry), W2 - Total leaf weight (dry)

EXCESISE 4: Identification of synthetic and natural plant harmones and growth retardants.

What is Plant hormones?

Plant hormones are small organic compounds that influence physiological responses to environmental stimuli at very low concentrations (generally less than 10-7M). Hormones are not directly involved in metabolic or developmental processes but they act at low concentrations to modify those processes.

According to Pincus and Thimann (1948), a plant hormone is defined as" organicsubstance produced naturally in the higher plants, controlling growth orother physiological functions at a site remote from its place of production and activein minute amounts."

What can they do?

Hormones regulate or influence a range of cellular and physiological processes, including

1. Cell Division
2. Cell Enlargement
3. Cell Differentiation
4. Flowering
5. Fruit Ripening
6. Movement (tropisms)

"Not all researchers agree that the term "hormone" should be applied to plants. "

Plants do not have a circulatory system and therefore hormone action in plants is fundamentally different from hormone action in animals. Many plant biologists use the term "plant growth regulator" instead of "hormone" to indicate this fact. The table below summarizes some of the differences between plant and animal
hormones.

Plant hormones	Animal hormones
1.Small molecules only	1.Peptides/proteins and/or small molecules
2. Produced throughout the plant	2. Produced in specialized "glands"
3. Mainly local targets (nearby cells and tissues)	3. Distant targets ("action at a distance")
4. Effects vary depending on interaction with other hormones	4. Specific effects
5."Decentralized" regulation	5. Regulation by central nervous system

The auxins were the first hormones to be discovered in plants and at one time considered to be the only naturally occurring plant growth hormones. Since then besides other less important hormones, two important groups of chemical substances having profound influence on the regulation of growth and development in plants have been discovered which are also considered as **natural plant growth hormones**. They are **gibberellins** and **cytokinins**. Beside these, **ethylene andabsicisic acid (ABA)** and more recently **brassinosteroids** have also acquired status of natural plant growth hormones.

Auxins:

The discovery of auxins dates back to last quarter of the 19th century when Charles Darwin was studying tropisms in plants. Went (1926) was successful in isolating this growth substance from Avena coleoptile tips which still retained the growth promoting activity. He cut off the tips of the Avena coleoptiles and placed them on small agarblocks for certain period of time and then placed the agar-blocks asymmetrically on cut coleoptile stumps. All the coleoptiles showed typical curvature even in dark. He also developed a method for determining the amount of this growth substance (i.e.auxin) which is active in very small amounts in the Avena coleptile tips. This method or the bioassay is famous by the name of Avena Curvature Test (Fig.4.1) .

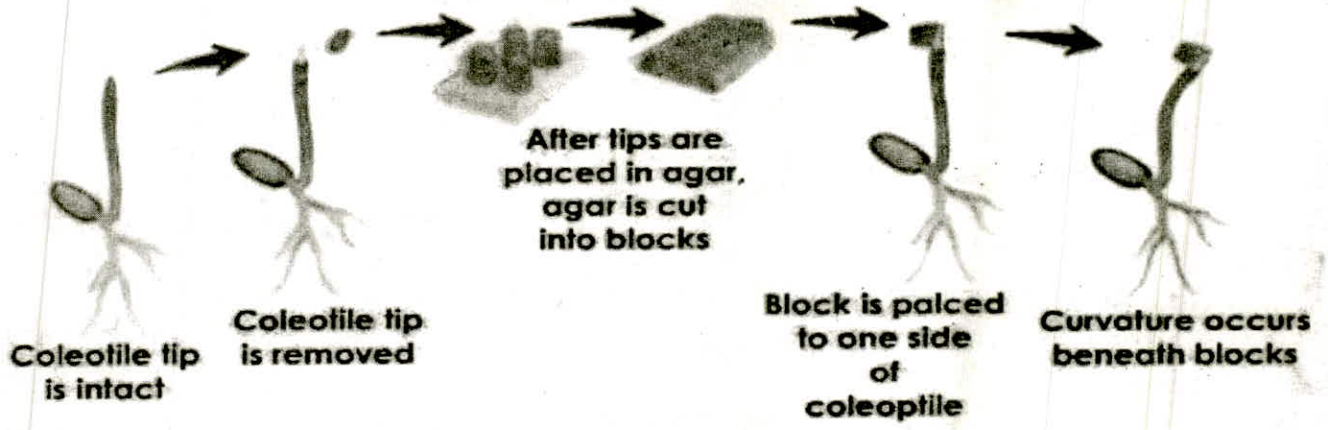


Fig.4.1 Avena Curvature Test.

Synthetic Auxins:

Auxin is a general term used to denote substances that promote the elongation of coleoptiles tissues, particularly when treated in the *Avena* coleoptiles test or in severalother bioassaytechniques. Indoleacetic acid is an auxin that occurs naturally in plants.Soon after the recognition of the importance of IAA as a plant hormone,compounds similar in structure were synthesized and tested for biological activity.Among the first compounds studied were substituted indoles, such as **indole-3-propionic acid** and **indole-3-butyric acid**. Both compounds are biologically active and commonlyused as rooting hormones in horticultural work. Both have the same indole rings as IAA and a terminal carboxyl group but differ in their side chains. If longer side chains are added to the indole ring, the compounds generally lack biological activity. Certain species of plants, however, possess enzymes capable of shortening the side chains and will convert the compounds to a biologically active molecule.Compounds lacking the indole ring but retaining the acetic acid side chain present in IAA are also biologically active.

Naphthaleneacetic acid is such a compound and it isused as a rooting hormone for certain plants. Another biologically active synthetic auxinlacking the indole ring is 2,4-dichlorophenoxyacetic acid. This compound, known as 2,4-D, is a potent auxin and is used as a weed killer. It is probably the most widely used of the synthetic auxins in commercial crop production. The carbamate compound was developed for use as a fungicide but was also found to have auxin activity. It lacks a ring structure but does possess an acetic acid side chain.

Physiological roles of Auxin: (Fig. 4.2)

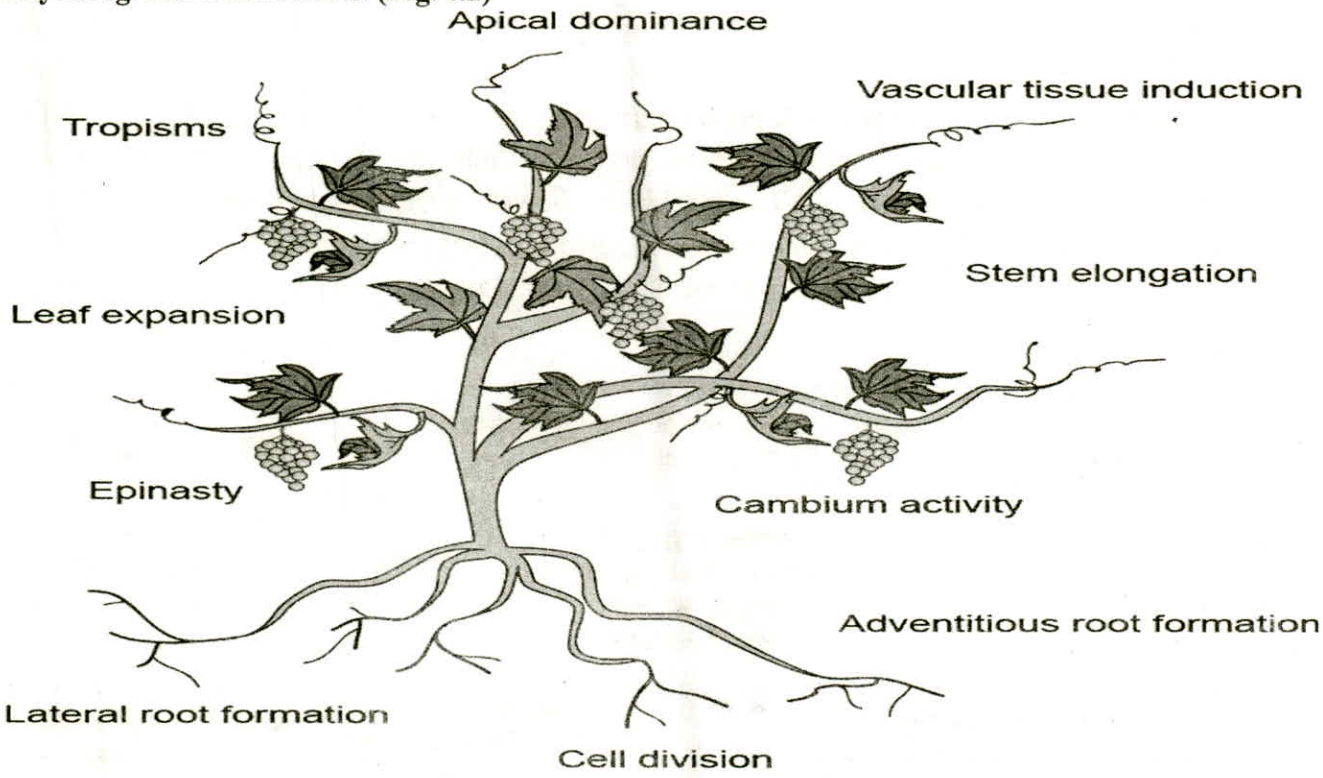


Figure 4.2 Summrized physiological role of Auxin

1. Cell Elongation

The primary physiological effect of auxin in plants is to stimulate the elongation of cells in shoot. A very common example of this can be observed in phototropic curvatures where the unilateral light unequally distributes the auxin in the stem tip (i.e. more auxin on shaded side than on illuminated side). The higher concentration of auxin on the shaded side causes the cells on that side to elongate more rapidly resulting in bending of the stem tip towards the unilateral light.

2. Apical Dominance

It has been a common observation in many vascular plants especially the tall and sparsely branched ones that if the terminal bud is intact and growing, the growth of the lateral buds just below it remained suppressed. Removal of the apical bud results in the rapid growth of the lateral buds. This phenomenon in which the apical bud dominates over the lateral buds and does not allow the latter to grow is called as apical dominance. Skoog and Thimann (1934) first pointed out that the apical dominance might be under the control of auxin produced at the terminal bud and which is transported downward through the stem to the lateral buds and hinders their growth. They removed the apical bud of broad bean plant and replaced it with agar block. This resulted in rapid growth of lateral buds. But, when they replaced the apical bud with agar block containing auxin, the lateral buds remained suppressed and did not grow (Fig. 4.3).

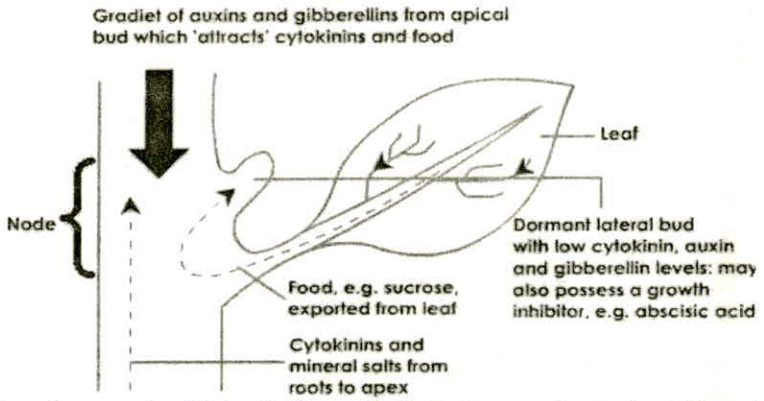


Figure 4.3 Possible Involvement of Plant Growth Substances in Apical Dominance in Presence of Apical Bud.

3. Root Initiation

In contrast to the stem, the higher concentration of auxin inhibits the elongation ofroot but the number of lateral branch roots is considerably increased i.e., the higher concentration of auxin initiates more lateral branch roots. Application of IAA in lanolin paste to the cut end of a young stem resulted in an early and extensive rooting. This fact is of great practical importance and has been widely utilized to promote root formation in economically useful plants which are propagated by cuttings.

4. Prevention of Abscission

Natural auxins have controlling influence on the abscission of leaves, fruits etc.

5. Parthenocarpy

Auxin can induce the formation of parthenocarpic fruits. In nature also, this phenomenon is common and in such cases the concentration of auxins in the ovaries has been found to be higher than in the ovaries of plants which produce fruits only after fertilization. In the latter cases, the concentration of the auxin in ovaries increases after pollination and fertilization (Figure 4.4).

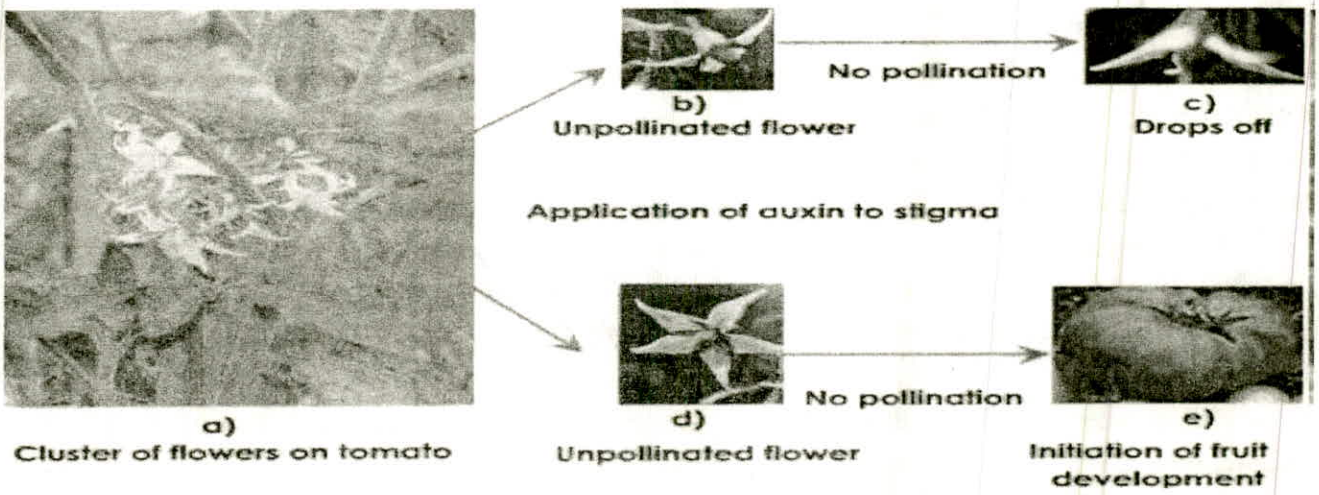


Figure 4.4 Auxin increase pollination and fertilization in developed ovaries

6. Respiration

It has been established that the auxin stimulates respiration and there is a correlation between auxin induced growth and an increased respiration rate. According to French and Beevers (1953), the auxin may increase the rate of respiration indirectly through increased supply of ADP (Adenosine diphosphate) by rapidly utilizing the ATP in the expanding cells.

7. Callus Formation

Besides cell elongation the auxin may also be active in cell division. In fact, in many tissue cultures where the callus growth is quite normal, the continued growth of such callus takes place only after the addition of auxin.

8. Vascular Differentiation

Auxin induces vascular differentiation in plants. This has been confirmed in tissue culture experiments and from studies with transgenic plants. Cytokinins are also known to participate in differentiation of vascular tissues and it is believed that vascular differentiation in plants is probably under the control of both auxin and cytokinins.

Gibberellins:

The discovery of gibberellins is quite fascinating and dates back to about the same period when auxins were discovered, but it was only after 1950s they came into prominence. A young Japanese scientist Kurosawa had been trying to find out why the rice seedlings infected by the fungus *Gibberella fujikuroi* (asexual stage *Fusarium monoliforme*) grew taller and turned very thin and pale. These are the symptoms of 'Backanae disease' (meaning foolish) which is known to Japanese for over a century. In 1926, he succeeded in obtaining a filtered extract of this fungus which could cause symptoms of the Backanae disease in healthy rice seedlings. In 1935, Yabuta isolated the active substance which was quite heat stable and gave it the name gibberellin.

Physiological roles of gibberellins

1. Seed Germination

Certain light sensitive seeds e.g. lettuce and tobacco show poor germination in dark.Germination starts vigorously if these seeds are exposed to light or red light. This requirement of light is overcome if the seeds are treated with gibberellic acid in dark.

2. Dormancy of Buds

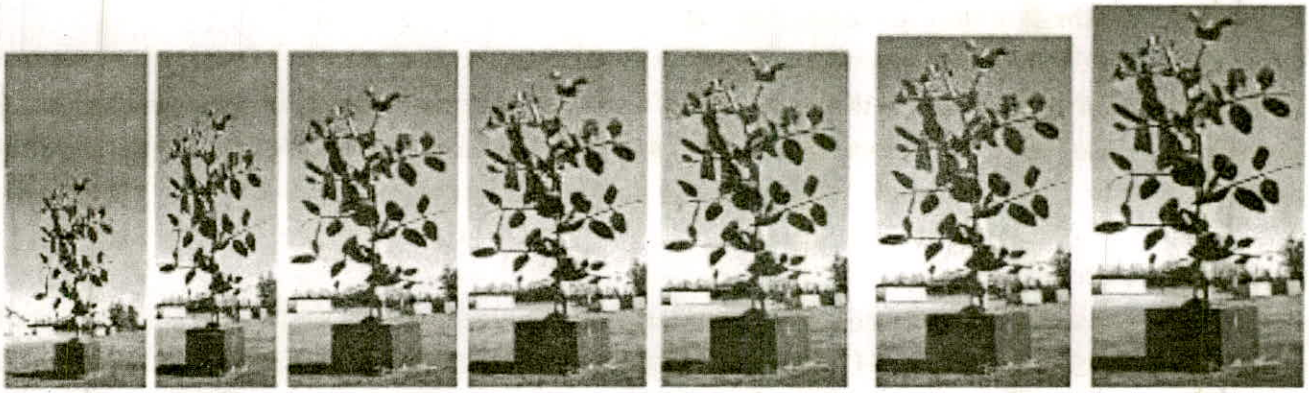
In temperate regions the buds formed in autumn remain dormant until next spring due to severe colds. This dormancy of buds can be broken by gibberellin treatment. In potatoes also, there is a dormant period after harvest, but the application of gibberellin sprouts the eyes vigorously.

3. Root Growth

Gibberellins have little or no effect on root growth. At higher concentration in some plants, however, some inhibition of root growth may occur. The initiation of roots is markedly inhibited by gibberellins in isolated cuttings.

4. Elongation of the Internodes

Most pronounced effect of gibberellins on the plant growth is the elongation of the internodes, so in plants such as dwarf pea, dwarf maize etc., they overcome the genetic dwarfism. For instance, the light grown dwarf pea plants have short internodes and expanded leaves. But, when treated with gibberellin the internodes elongate markedly and they look like tall plants (Fig 4.5).



The influence of gibberellic acid(GA) on the growth of variety Meteor drarf pea. The plant on the left received no GA and shows the typical dwarf habit. The remaining plants were treated with GA; the dose per plant in micrograms is shown. With doses up to 5 micrograms there is increased growth of the stems with increase in GA dosage. This is the principle of the dwarf pea assay of gibberellins.

Figure 4.5 Effect of GA on the growth of dwarf variety of Pea.

It is considered that in such dwarf plants (i) the gene for producing gibberellins is missing, or (ii) the concentration of the natural inhibitors is higher. When external gibberellins are applied, the deficiency of the endogenous gibberellins is made good or the external gibberellins overcome the effect of natural inhibitors which fall short.

5. Bolting and Flowering

In many herbaceous plants the early period of growth show rosette-habit with short stem and cauline leaves. Under short days the rosette habit is retained while under long days bolting occurs i.e., the stem elongates rapidly and is converted into floral axis bearing flower primordia. This bolting can also be induced in such plant e.g. Rudbeckia speciosa (Long Day Plant) by the application of gibberellins even under noninductive short days. In Hyoscyamus niger (Long Day Plant) gibberellins treatment causes bolting and flowering under non-inductive short days.

6. Parthenocarpy

Germination of the pollen grains is stimulated by gibberellins, likewise the growth of the fruit and the formation of parthenocarpic fruits can be induced by gibberellins treatment. In many cases e.g. pome and stone fruits where auxins have failed to induced parthenocarpy the gibberellins have proven to be successful. Seedless and fleshy tomatoes and large sized grapes are produced by gibberellins treatment on commercial scale.

7. Light Inhibited Stem Growth

Treatment of light grown plants with gibberellins also stimulates the stem growth and they appear to be dark grown. In such cases the protein content of the stem falls while soluble nitrogen content increases probably due to more breakdowns of proteins than their synthesis.

8. De nova Synthesis of the Enzyme-∝-Amylase

One of the important functions of gibberellins is to cause de novo synthesis of the enzyme a-**amylase** in the **aleurone layer** surrounding the endosperm of cereal grains during germination. This enzyme brings about hydrolysis of starch to form simple sugars which are then translocated to growing embryo to provide energy source.

Cytokinins:

The discovery of kinetin is comparatively more recent. Its credit goes to Miller et al (1950) who were working in Prof. Skoog's lab at the University of Wisconsin on the growth of tobacco pith callus in culture and wanted it to grow indefinitely. Some of the very important and commonly known naturally occurring cytokinins are as follows.

(A) Zeatin

Zeatin is the most abundant and widely distributed natural cytokinin in higher plants and in some bacteria. Although this cytokinin was known earlier but it was obtained in pure crystalline form in 1963 by Letham from immature corn grains and named as Zeatin. It was identified as 6-(4-hydroxy-3-methylbut-trans-2enyl) amino purine by Letham et *al.* (1964) and was synthesized by Shaw and Wilson (1964).

- Zeatin exhibits strong kinetin like activity in stimulating plant cell to divide in presence of auxin in culture media.
- Zeatin resembles kinetin in molecular structure because both are adenine or amino purine derivatives.
- Zeatin is remarkably more active than any other cytokinin probably because of the presence of a highly reactive allylic-OH group in its side chain.

Synthetic cytokinins

Some synthetic chemical compounds which show cytokinin activity but have not been isolated from plants are known. Benzyl adenine (BA) is one such example. Although there are a few reports of this compound in plants but it's uncommon in plants and is largely a synthetic cytokinin. Another synthetic cytokinin is thidiazuron that is used commercially as defoliant and a herbicide.

Physiological roles of Cytokinins

1. Cell division

One of the important biological effects of kinetin on plants is to induce cell division in the presence of sufficient amount of auxin (IAA), especially in tobacco pith callus, carrot root tissue, soybean cotyledon, pea callus etc.,

2. Cell enlargement

Like auxins and gibberellins, the kinetin may also induce cell enlargement. Significant Cell enlargement has been observed in kinetin treatment in leaf discs cut from etiolated leaves of *Phaseolus vulgaris*, pumpkin cotyledons, tobacco pith cultures, cortical cells of tobacco roots excised Jerusalem artichoke tissue etc.,

3. Initiation of inter-fascicular cambium

Kinetin can induce formation of inter - fascicular cambium. This has in fact been shown by Sorokin et *al.* (1962) in pea stem sections.

4. Morphogenesis

Kinetin also has ability to cause morphogenetic changes in an otherwise undifferentiated callus. For instance the tobacco pith callus can be made to develop either buds or roots by changing the concentration of kinetin and auxin.

5. Counteraction of apical dominance

Cytokinins play a role in initiating the growth of lateral buds has also been proved by physiological studies made on cytokinin overproducing mutants of tobacco.

6. Dormancy of seeds

Like gibberellins, the dormancy of certain light sensitive seeds such as lettuce and tobacco can also be broken by kinetin treatment in dark.

7. Delay of senescence:

The ageing process of the leaves usually accompanies with loss of chlorophyll and rapid breakdown of proteins. This is called senescence. Richmond and Lang showed that this senescence could be postponed to several days in detached Xanthium leaves by kinetin treatment.

8. Promotion of chloroplast development

Cytokinins are known to enhance conversion of etioplasts into chloroplast when etiolated seedlings after treatment with cytokinins are exposed to light. In such cases, the chloroplasts develop extensive grana and chlorophylls and the rate of synthesis of photosynthetic enzymes is much greater in comparison to those etiolated seedlings which are illuminated without cytokinin treatment.

Brassinosteroids:

Brassinosteroids (BRs) are a class of polyhydroxysteroids that have been recognized as a sixth class of plant hormones. These were first explored nearly forty years ago when Mitchell et al. reported promotion in stem elongation and cell division by the treatment of organic extracts of rapeseed (Brassica napus) pollen Brassinolide was the first isolated brassinosteroid in 1979 when it was shown that pollen from Brassica napus could promote stem elongation and cell divisions, and the biologically active molecule was isolated.

Physiological roles of brassinosteroids

(A) Cell level

- Stimulation of elongation and fission
- Effect on hormonal balance
- Effect on enzyme activity; H.-pump activation
- Activation of protein and nucleic acid synthesis

- Effect on the protein spectrum and on the amino acid composition of proteins
- Effect on the fatty acid composition and on the properties of membrane
- Enhancement of the photosynthetic capacity and of translocation of products

Stimulation

(A) Whole plant level

- Growth promotion
- Increase in the success of fertilization
- Shortening the period of vegetative growth
- Size and quantity of fruits increase
- Effect on the content of nutritive components and fruit quality improvement
- Increased resistance to unfavourable environmental factors, stress and diseases

Abscisic Acid (ABA)

In 1963, a substance strongly antagonistic to growth was isolated by **Addicott** from young cotton fruits and named **Abscisin II**. Later on, this name was changed to **Abscisic acid (ABA)**. The chemical name of abscisic acid is 3-methyl 5-1' (1'-hydroxy, 4-oxy-2', 6', 6'-trimethyl2-cyclohexane-1-yl) - cis, trans-2, 4 penta-dienoic acid.

Physiological roles of ABA

1. Stomatal regulation

The role of ABA in causing stomatal closure in plants undergoing water-stress is now widely recognized. The application of exogenous ABA causes closing of stomata by inhibiting the ATP mediated H+/K+ ions exchange pumps in guard cells.

2. Leaf abscission

ABA is known to produce abscission layers at the base of the leaf petiole where dead cells are formed. ABA production increases in senescing leaves once the photosynthetic activity of the leaves decreases below the compensation point.

3. Seed and bud dormancy

Seeds and buds remain dormant to ward off unfavourable seasonal and soil conditions for germination and growth respectively. Presence of ABA in such seeds and buds provides dormancy to these structures. Once favourable conditions are available, ABA gets denatured or overcome by production of growth promoting hormones such as GA or IAA.

4. Other Functions

Process of tuberization, fruit ripening, increasing the resistance of temperate zone plants to frost injury, inhibition of GA-induced synthesis of $\propto$ amylase in aleurone layers of germinating barley, inhibition of precocious germination and vivipary and increase in root: shoot ratio at low water potentials.

Ethylene:

Neljubow in 1901 identified ethylene in laboratory air from illuminating coal gas which caused typical symptoms in etiolated pea seedlings grown in dark in the lab, *viz.*,

(i) inhibition of stem elongation, (ii) stimulation of radial swelling of stems and (iii) horizontal growth of stems with respect to gravity.

Physiological role of ethylene

1. Fruit Ripening

One of the most pronounced effects of ethylene is in ripening of fruits and therefore, ethylene is also known as fruit ripening hormone.

2. Plumular Hook Formation

In etiolated dicot seedlings, the plumular tip (i.e., shoot apex) is usually bent like a hook. This hook shape is advantageous to seedling for penetration through the soil, protecting the tender apical growing point from being injured.

3. Triple Response

Ethylene causes 'triple response' of etiolated seedling such as in pea which consists of (i) inhibition of stem elongation, (ii) stimulation of radial swelling of stems and (iii) horizontal growth of stems with respect to gravity (i.e. diageotropism)

4. Formation of Adventitious Roots and Root Hairs

Ethylene induces formation of adventitious roots in plants from different plant parts such as leaf, stem, peduncle and even other roots. In many plants especially Arabidopsis, ethylene treatment promotes initiation of root hairs.

5. Inhibition of Root Growth

Ethylene is known to inhibit linear growth of roots of dicotyledonous plants.

6. Leaf Epinasty

When the upper side (adaxial side) of the petiole of the leaf grows faster than the lower side (abaxial side), the leaf curves downward. This is called as epinasty. Ethylene causes leaf epinasty in tomato and other dicot plants such as potato, pea and sunflower.

7. Flowering

Ethylene is used commercially to synchronize flowering and fruit set in pineapple.

8. Sex Expression

In monoecious species especially some cucurbits like, cucumber, pumpkin, squash and melon, ethylene strongly promotes formation of female flowers thereby suppressing the number of male flowers considerably.

9. Senescence

Ethylene enhances senescence of leaves and flowers in plants. During senescence, concentration of endogenous ethylene increase with decrease in concentration of cytokinins and it is now generally held that a balance of these two phytohormones controls senescence.

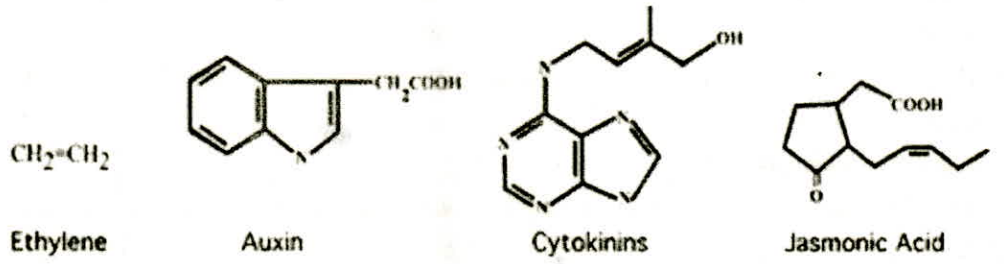
10. Abscission of leaves

Ethylene promotes abscission of leaves in plants. Older leaves are more sensitive than the younger ones. Activity of cellulases enzymes results in cell wall loosening and cells separation ultimately leading to leaf abscission.

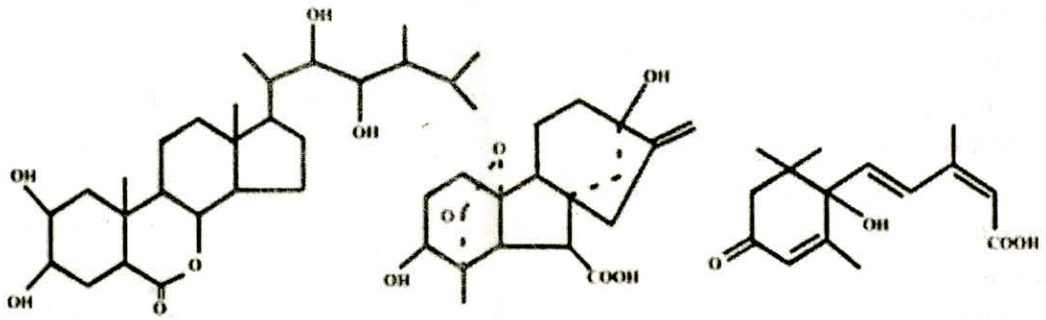
11. Breaking Dormancy of Seeds and Buds

Ethylene is known to break dormancy and initiate germination of seeds. Seed dormancy is overcome in strawberry, apple and other plants by treatment with ethylene.

Chemical structures of different plant hormones



Ethylene Auxin Cytokinins Jasmonic Acid



Brassinosteroids Gibberellins Abscisic Acid

Growth Retardants:

The plant hormones or regulators, which inhibit or retard growth and development of plants are called as growth retardants. The major growth regulators used are as follows:

1. Maleic hydrazide

Physiological roles of MH

- Inhibition of seed germination
- Induction of dwarfing effect.
- Stimulates branching and lateral shoot growth and prevents apical dominance
- Prolonged bud dormancy
- Prevents flowering in short day plants
- Prevents sprouting of onions and potatoes during storage

2. Jasmonic acid

Physiological roles of JA

- It acts as a growth inhibitor and seems to participate in leaf senescence and in the defense mechanism against fungi.
- Jasmonate derivatives induce the accumulation of so-called jasmonate-inducedproteins that were found in all plant species tested. Their accumulation can also be caused by desiccation or ABA effects.
- Jasmonate induced proteins are lacking in roots, in bleached leaves, and in leave of chlorophyll deficient Hordeum vulgare mutants.

Influence in fruit culture

- n-Propyl dihydrojasmonate (PDJ) treatment at 91 days after full bloom (DAFB) decreased endogenous ABA and its metabolite but increased ethylene concentration and hence increased fruit ripening of mangoes (Kondog et al.,2004).
- Methyl jasmonate treatments increased ethylene production at the climacteric stage and was more pronounced at a higher concentration (10-3M) of applied methyl jasmonate. Skin colour of ripe fruit was significantly improved with exogenous application of methyl jasmonate .
- Raspberries treated with MJ had higher soluble solids content, total sugars, fructose, glucose, sucrose and lower titratable acids (TAs), malic acid and citricacid than untreated fruit. MJ also significantly enhanced the content of flavonoids and the antioxidant capacities in the fruit (Wang et al., 2005).

3. Uniconazole

Influence in fruit culture

- Retards bolting in radish and flowering in pear when it is applied before floral initiation
- Control internode length elongation in raspberries

4. Paclobutrazol

Mode of action

Suppression of growth by paclobutrazol occurs because the compound blocks three steps in the terpenoid pathway for the production of gibberellins by binding with and inhibiting the enzymes that catalyze the metabolic reactions

Influence in fruit culture

- Treated trees of apple have more compact crowns and some what smaller and darker green leaves, but otherwise look normal. The amount of shoot growth reduction ranges from a low of 10% to a high of 90%, with average growth reduction being 40-60% when recommended dose rates are applied.
- Increased concentration of paclobutrazol (1000 ppm) suppressed the root length and increased the root diameter of Assam lemon. Paclobutrazol-treated plants showed better survival at the nursery stage than control.(Singh et al., 2000)
- Paclobutrazol increased root:shoot ratio.
- Paclobutrazol applications stimulate flowering two months after the application, or 2 months earlier than natural flowering. The application increases fruit production by as much as 73 -142 %.
- Trees treated with paclobutrazol generally have leaves with a rich green colour suggesting high chlorophyll content. Paclobutrazol treatment, which blocks the production of gibberellins, results in a shunting of the intermediate compounds from gibberellin synthesis to the production of more leaves.
- Treatment with paclobutrazol promotes the production of abscisic acid - cause stomates to close, reducing water loss from leaves through transpiration.

Exercise 5: Bio-assay and biosynthesis of phytoharmones.

1. Bioassay for auxins:

Several, bioassays have been devised for auxins such as *Avena* **curvature** test, Avena section test, split pea stem curvature test, cress root inhibition test etc. A brief account of the Avena curvature test is given below:

***Avena* Curvature Test**

Principle

This test is based on the polar transport of the auxin in *Avena coleoptiles*. The auxin applied on one side of the cut coleoptiles stump will diffuse down that side only and will cause that side to grow more resulting in curvature of the coleoptiles. Within limits this curvature is directly proportional to the amount of auxin applied.

Procedure

i. Avena grains germinated and grown in total darkness. The seedlings are exposed to short periods (2-4 hrs) of red light two days after germination.
ii. When the roots are about 2mm. Long, the seedlings are planted in special glass holders, using the water culture method.
iii. The straight coleoptiles are selected.
iv. The tips of the coleoptiles (about 1 mm) are removed and placed on agar-agar.
v. The agar is cut into blocks of standard size (usually 1 mm3) which now contain auxin.
vi. After about 3 hours a second decapitation of the coleoptiles is made to remove the tip which might have regenerated and the first leaf of the seedling is pulled so that its connection from the base is broken.
vii. An agar block containing auxin is now placed on one side of the cut coleoptile. The projecting primary leaf gives support to the auxin block.
viii. After about 90 minutes the shadowgraphs of the seedlings are taken and the angle of curvature (a) is measured by drawing a vertical line and a line parallel to the curved portion of the coleoptiles.
ix. Within limits the curvature of the coleoptile is directly proportional to the concentration of auxin in agar block. In case of Indole-3-Acetic Acid (IAA) the maximum response is at about 0.2 mg/ litre.

Biosynthesis of Auxin :

Tryptophan dependent pathways

In 1935, Thimann demonstrated that a fungus Rhizopus suinus could convert an amino acid tryptophan (trp) into indole-3 acetic acid (IAA). Since then, it is generally held that tryptophan is primary precursor of IAA in plants.The indole-3-acetic acid (IAA) can be formed from tryptophan by 3 different pathways (Fig.5.1).

(a) TAM (Tryptamine) pathway

Tryptophan is decarboxylated to form tryptamine (TAM) followed by deamination of the latter resulting in the formation of indole-3-acetaldehyde (IAld). The enzymes involved are tryptophan decarboxylase and tryptamine oxidase respectively. IAId is readily oxidised to indole-3-acetic acid (IAA) by the enzyme IAId dehydogenase.

(b) IPA (Indole-3-pyruvic acid) pathway

Tryptophan is deaminated to form indole-3-pyruvic acid (IPA) followed by decarboxylation of the latter resulting in the formation of indole-3-acetaldehyde (IAId). The enzymes involved are tryptophan transminase and indole pyruvate decarboxylase.

(c) IAN (Indole-3-acetonitrile) pathway

It occurs in some plants especially those belonging to families Brassicacease, Poacceae and Musaceae. Tryptophan is converted into IAA in thepresence of the enzyme nitrilase. Indole-3-acetaldoxime and indole-3-acetaonitrile (IAN) are the intermediates.

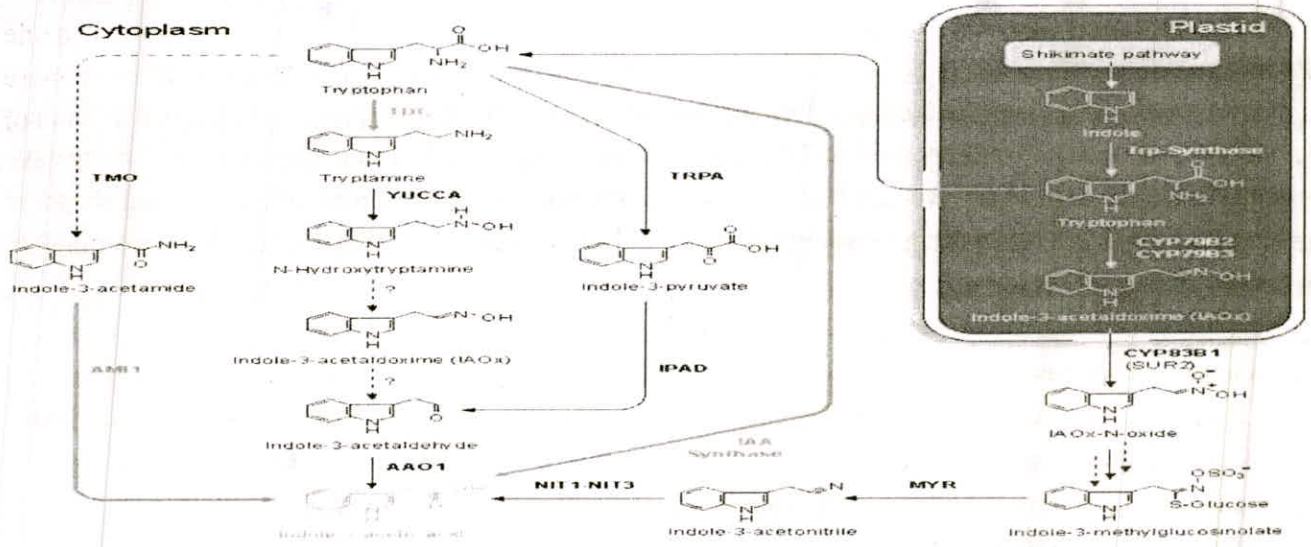


Figure 5.1 Pathways of tryptophan-dependent indole-3-acetic acid biosynthesis

The abbreviations are

AAO1 = indole-3-acetaldehyde oxidase, **AMI1** = amidase 1 (indole-3-acetamide hydrolase), **IAOx** = N oxide: indole-3-acetaldoxime-N-oxide, **IPAD** = indole-3-pyruvic acid decarboxylase; **MYR** =myrosinase, **NIT1-3**=nitrilases isogenes 1 -3, **TDC**= tryptophan decarboxylase, **TMO**= tryptophan-2-monooxygenase, **TRPA** = tryptophan aminotransferase, **YUCCA** = flavin monooxygenase-like protein.

Transport of auxin in plant

The transport of auxin in plant is predominantly polar. In stems, polar transport of auxin is basipetal *i.e.*, it takes place from apex towards base. In roots also, the auxin transport is polar but is primarily acropetal. Jacobs (1961) found polar transport of auxin in coleus stem sections to be both basipetal and acropetal in the ratio of 3:1. According to Audus (1959) some of the auxin synthesized by leaves may be transported to other plant parts through phloem in a rather non-polar manner. Phototropic and geotrophic movements indicate towards lateral transport of auxins in stem tip and root tip respectively.

2. Bioassay for gibberellins

A number of bioassays are known for gibberellins such as pea test, dwarf corn test, lettuce hypocotyls test, cucumber hypocotyls test, barley endosperm test, ect. The relative activity of the different gibberellins is different in different bioassay systems. For instance, the relativity of GA1 in dwarf corn (strains d1, d3 and d5) is in the following order:-

d1 - GA1>GA3>GA4>GA7>GA5>GA6>GA8>GA9
d3 - GA5>GA7>GA9>GA3>GA4>GA1>GA6>GA2
d5 - GA5>GA7>GA3>GA4>GA9>GA1>GA2>GA2>GA8

A brief account of the dwarf corn test is given below:-

Principle

This bioassay is based on the fact that gibberellins cause elongation of the internodes, and in case of corn they also increase the length of the leaf sheaths which surround the internodes.

Procedure

A measured amount of the test solution in a suitable wetting agent is applied into the first unfolding leaf of corn seedlings when they are 6 to 7 days old. The seedlings are allowed to grow for 6 or 7 more days till the first and the second leaves are fully developed. The increase in the length of the first leaf sheath is measured and is plotted against the concentration of gibberellins applied. Within limits, a direct relationship is observed between the two.

Biosynthesis of gibberellins :

In plants GAs are biosynthesized in apical tissues and there are three main sites of their biosynthesis,

(i) Developing seeds and fruits,

(ii) Young leaves of developing apical buds and elongating shoots and

(iii) The apical regions of roots.

The pathway of GA biosynthesis can be divided into three stages each of which is accomplished in a different cellular compartment.

Stage I. Formation of terpenoid precursors and ent-kaurene in plastids.

GA is biosynthesized from a 5-C precursor IPP. The IPP may be synthesized either in plastids or cytosol. From IPP, 10-C (GPP), 15-C (FPP) and 20-C (GGPP) precursors of terpenoids are formed by condensation of 5-C units (IPP). After the formation of GGPP, the pathway becomes specific for GAs. GGPP is converted by two cyclization reactions through copalyl pyrophosphate into entkaurene. These reactions are catalysed by the enzymes cyclases which are located in proplastids and not in mature chloroplasts and infact constitute the first step that is specific for GAs. This step of GA biosynthesis is inhibited by compounds such as Amo-1618, Phosphon D and CCC.

Stage II. Oxidations to form GA12 and GA53 on ER through GA12 aldehyde.

The **ent -kaurene** is transported from plastids to **ER (endoplasmic reticulum)**. Now a methyl group on ent-kaurene at 19th-carbon position is oxidized to carboxylic group which is followed by contraction of ring B from 6-C to 5-C ring structure to form **GA12 aldehyde**. GA12 aldehyde is subsequently oxidized to give **GA12 which is precursor to all other GAs in plants**. Hydroxylation of GA12 at C-13 results in the formation of GA53. The enzymes catalyzing the above oxidation reactions are mono-oxygenases which are located on ER and utilize cytochrome P450 in these reactions. Activity of these enzymes is inhibited by paclobutrazol and other inhibitors before GA12 -aldehyde.

Stage III. Formation of all other GAs from GA12 or GA53 in cytosol.

All other steps in the biosynthesis of GAs from GA12 of Ga53 are carried out in cytosol by soluble enzymes called dioxygenases. These enzymes require molecular O2 and 2-oxoglutarate as cosubstrates and use ferrous iron (Fe++) and ascorbic acid as cofactors.Environment factors such as temperature and photoperiod are known to affect biosynthesis of gibberellins.

Biosynthetic pathway of GA

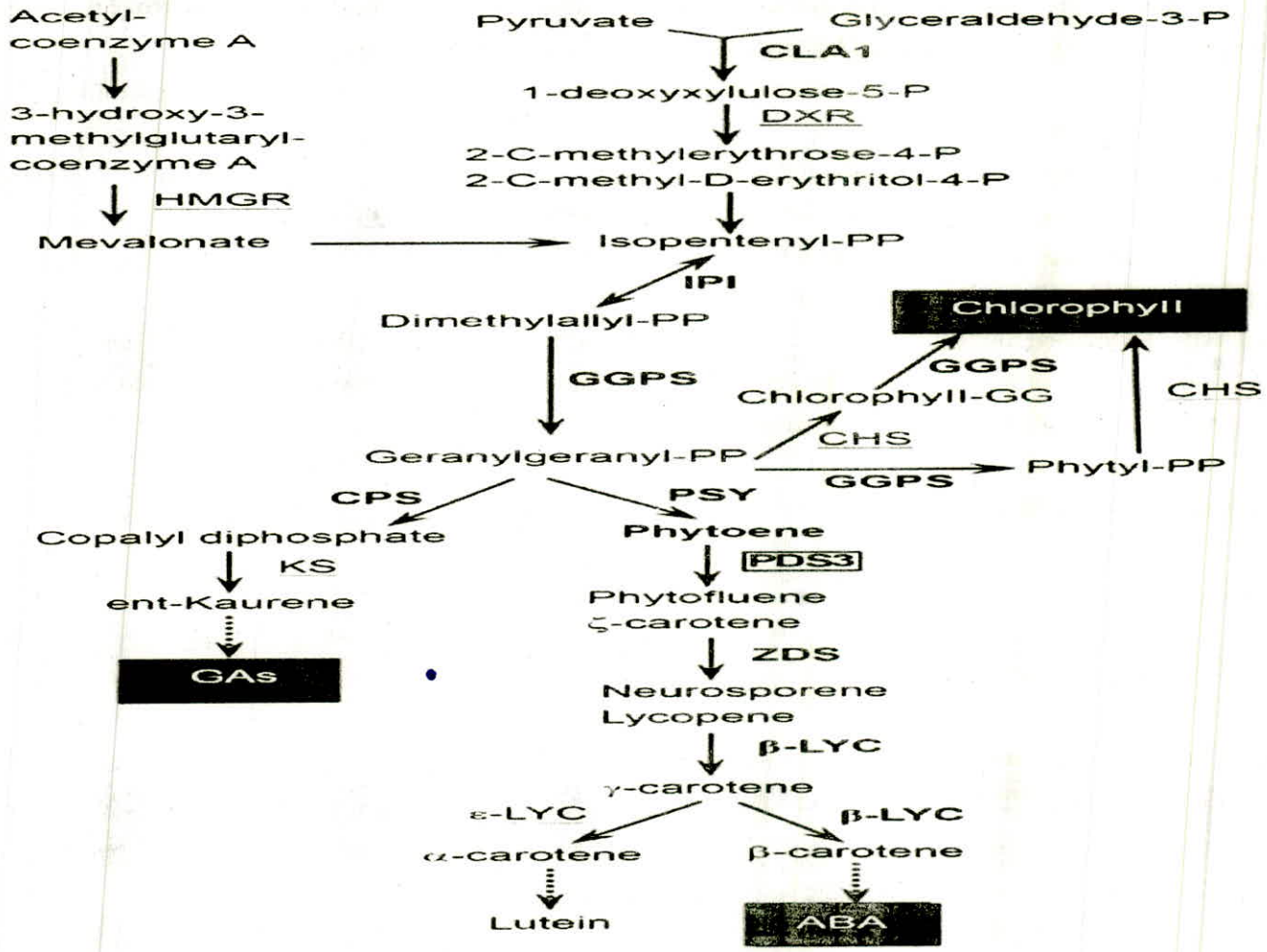


Figure 5.2 Biosynthetic pathway of GA

Gibberellins transport in plant

Gibberellins have been found from both phloem and xylem exudates from a variety of plants. Unlike auxins, the transport of gibberellins in plants is non-polar. It is believed that gibberellins are translocated through phloem according to a flow pattern which is similar to those of carbohydrates and other organic solutes. However, gibberellins transport may also occur in xylem due to its lateral movement between the two vascular tissues i.e.,xylem and phloem. The gibberellins are not translocated in plantas free molecules but probably in their bound form as gibberellins-glycosides.

(3) Bioassay for cytokinins :

A number of bioassays have also been devised for cytokinins which are based on their specific physiological activities. They are Cell division tests, Chlorophyll retention tests, Cell enlargement tests, Germination tests and differentiation tests. A brief account of one of the cell division tests which are based on the induction of cell division in cytokinin requiring tissue cultures is given below:

Carrot Root Phloem Bioassay

i. Mature roots of cultivated carrot (Daucus carota var. sativus) are peeled and the surface sterilized.
ii. With the help of a special cutter they are cut into thin slices about 1-2 mm. thick
iii. With the help of a canula, secondary phloem explants are removed from a distance about 1.2 mm. away from the cambium
iv. The secondary phloem explants weigh about 2.5-3 mg.
v. About three explants are inoculated into a culture tube containing 10 ml. of medium.
vi. The culture tubes are placed on a wheel which turns of a horizontal axis at 1 rpm. so that the explants are alternatively exposed to medium and the air.
vii. After a specified time (about 18-21 days) the explants are removed, weighed, macerated and the cells are counted. The number of the cells is converted into milligrams (1mg being approximately equal to 10,000 cells) and is plotted against the time in days.

Biosynthesis of cytokinins

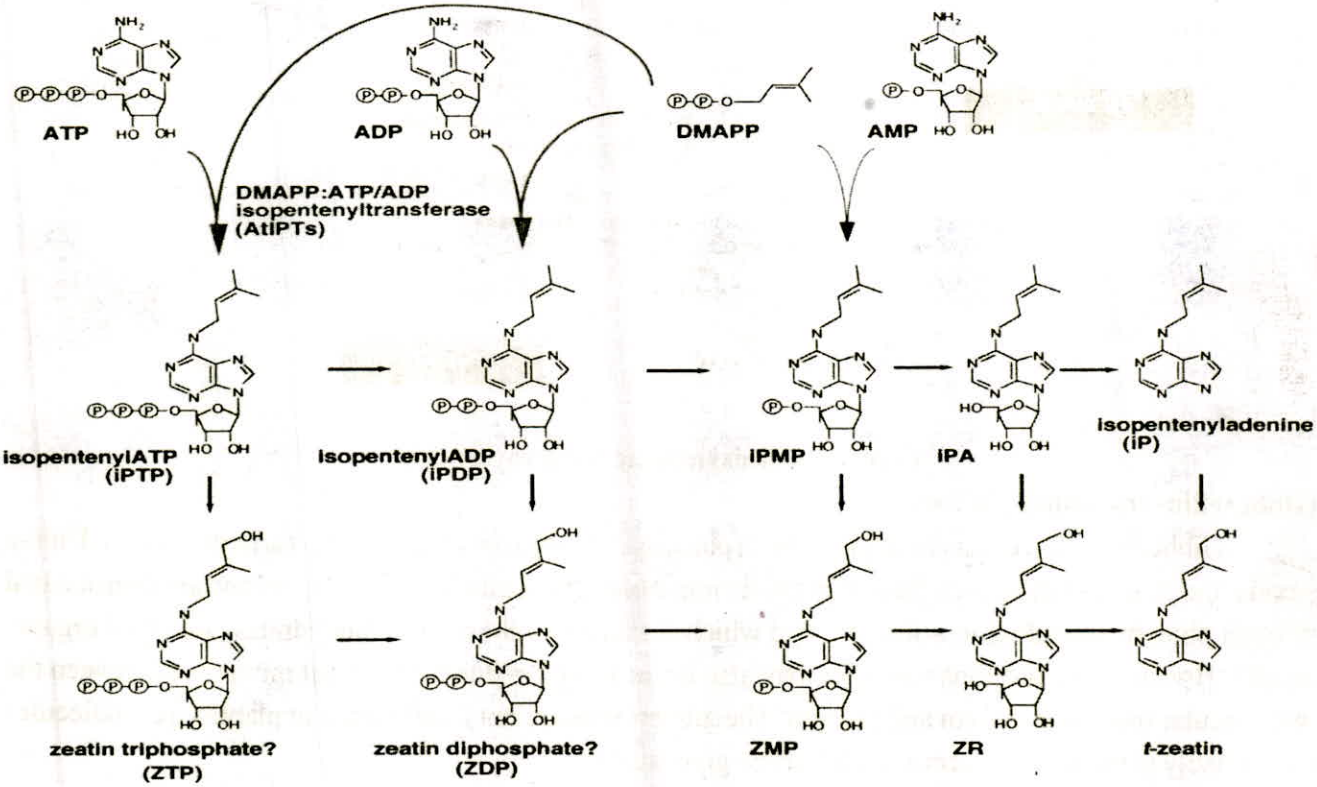


Figure 5.3 Biosynthetic pathway of cytokinins

(4) Biosynthesis of Brassinosteroids :

The BR is biosynthesized from campesterol. The biosynthetic pathway was elucidated by Japanese researchers and later shown to be correct through the analysis of BR biosynthesis mutants in Arabidopsis thaliana, tomatoes, and peas. The sites for BR synthesis in plants have not been experimentally demonstrated. One well-supported hypothesis is that all tissues produce BRs, since BR biosynthetic and signal transduction genes are expressed in a wide range of plant organs, and short distance activity of the hormones also supports this. Experiments have shown that long distance transport is possible and that flow is in an acropetal direction, but it is not known if this movement is biologically relevant. Brassinosteroids are recognized at the cell membrane, although they are membrane-soluble.

BRs have been shown to be involved in numerous plant processes:

- Promotion of cell expansion and cell elongation; works with auxin to do so.
- It has an unclear role in cell division and cell wall regeneration.
- Promotion of vascular differentiation; BR signal transduction has been studied during vascular differentiation.
- Necessary for pollen elongation for pollen tube formation.
- Acceleration of senescence in dying tissue cultured cells and delayed senescence.
- Provide some protection to plants during chilling and drought stress.
- Application of brassinosteroids to cucumbers was demonstrated to increase the metabolism and removal of pesticides, which could be beneficial for reducing the human ingestion of residual pesticides from non organically grown vegetables.

(5) Biosynthesis of ABA:

Extensive studies done by researchers with ABA deficient mutant of tomato, Arabidopsis and other plants have clearly shown that ABA is synthesized in higher plants not from simple terpenoid precursors directly through 15-C farnesyl diphosphate (FPP), but indirectly through carotenoid pathway as breakdown product of 40-C xanthophylls such as violaxanthin or neoxanthin (Figure 5.4).

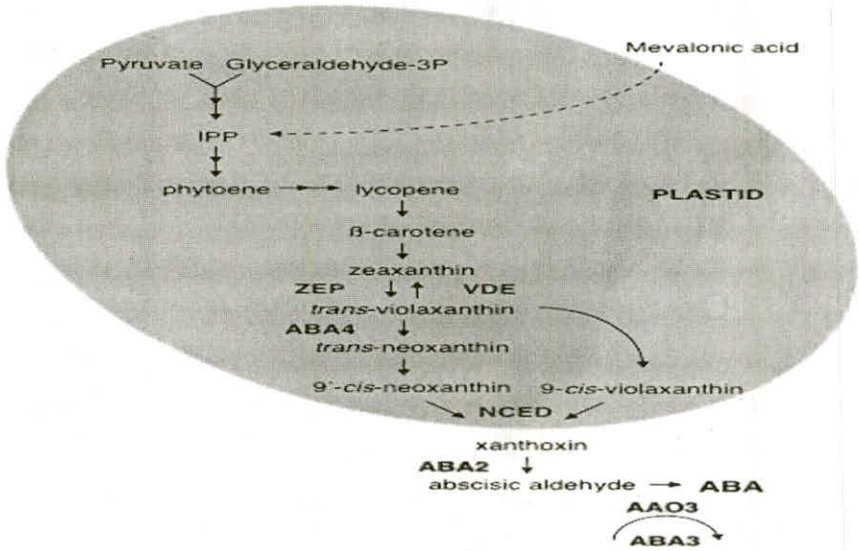


Figure 5.4 Biosynthetic pathway of ABA

ABA transport in plant

ABA is synthesized in all types of cells that contain chloroplasts or other plastids. It occurs predominantly in mature green leaves. The concentration of ABA in specific plant tissues varies greatly at different developmental stages or in response to environmental conditions especially water stress. The concentration of ABA in plant tissue is regulated by (i) its synthesis, (ii) degradation, (iii) compartmentation and (iv) transport.In plant, ABA predominantly occurs in its free form but it may also occur in conjugated form as glycoside with some simple sugar molecule such as glucose forming ABA-?-D-glucosyl ester. ABA is biologically inactive in its conjugated or bound form.

Biosynthesis of ethylene:

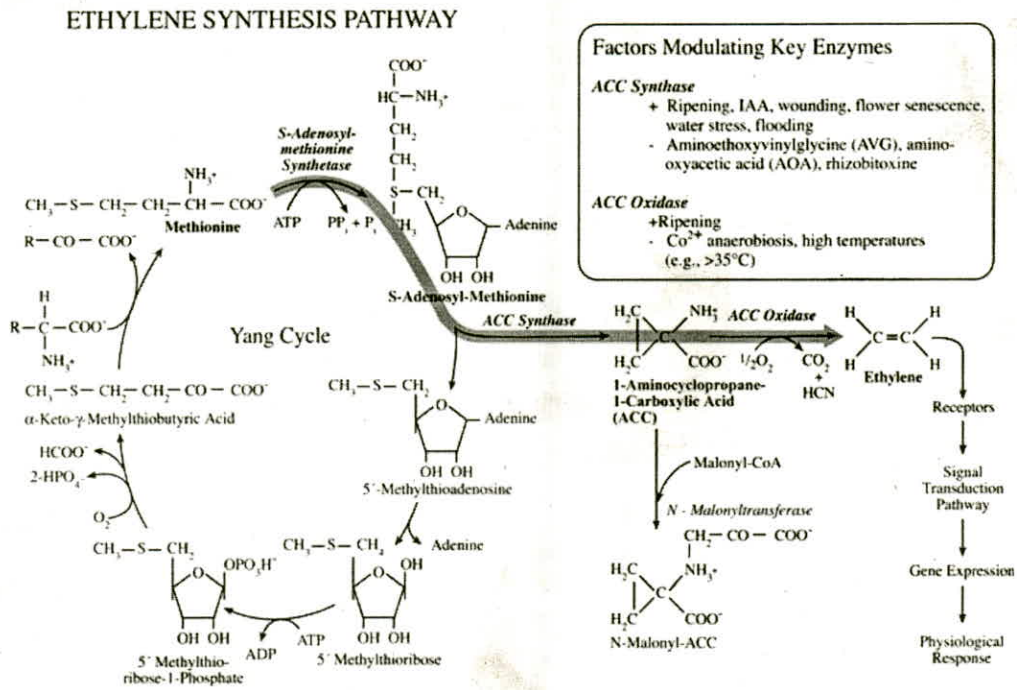


Figure 5.5 Biosynthesis pathway of ethylene

The process of ethylene biosynthesis is a three steps pathway :

(A) First Step : In the first step, an adenosine group (i.e.adenine+ribose) is transferred to methionine by ATP to form S-adenosylmethionine (SAM), This reaction is catalysed by the enzyme SAM-synthetase (methionine adenosyltransferase).

(B) Second Step : In the second step, SAM is cleaved to form 1-aminocyclopropane-1-carboxylic acid (ACC) and 5'-methylthioadenosine (MTA) by the enzyme ACC-synthase. Synthesis of ACC is rate limiting step in ethylene biosynthesis in plant tissues. Exogenously supplied ACC greatly enhance production of ethylene in plant tissues.

(C) Third Step : In the third and last step of ethylene biosynthesis, ACC is oxidized by the enzyme ACC oxidase (previously called ethylene forming enzyme i.e, EFE) to form ethylene. Two molecules, in each of HCN and H2O are eliminated. ACC oxidase activity can be rate limiting step in ethylene biosynthesis in plant tissues which show high rate of ethylene production such as ripening fruit.The enzyme ACC oxidase requires ferrous iron (Fe^{2+}) and ascorbate as cofactors.ACC can be conjugated to give N-malonyl ACC and thus, may play an important role is regulation of ethylene biosynthesis.

Exercise 6: Preparation and application of plant growth regulator solutions for propagation.

Materials Required: Plant growth regulator(s), measuring cylinder, volumetric flask, beaker(s), electronic balance, distilled water.

Preparation of PGRs solution:

- 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.
- For every use one should prepare fresh solution.

What is solutions?

Solutions are systems in which one component (the solute) is dispersed throughout the other (the solvent) in the form of molecules or ions. Theoretically, the solvent may be a gas, a solid,or a liquid, but solutions in which the solvent is a liquid are by far the most important in living organisms. Water is practically the only solvent that functions in plants, but the solutes concerned are numerous and includes all soluble inorganic salts that may be present in the soil, as well as oxygen, CO^2, sugars, organic acids and various other soluble organic compounds. Standard solution contains a known weight of the substances dissolved in a known volume of solvent. The term concentration denotes the proportion of solutes and solvents in a solution.

There are numerous ways in which concentration and composition of a solution may be expressed.

1. Per cent solution

Expressed in terms of percentage. In this type of a solution a known unit of the solute is dissolved in 100 units of water. When the solute is a solid and the solvent a liquid, a per cent solution is prepared on a W/V basis, i.e., a known wt of a substance (solute) is added to 100 ml of the solvent.

(A) Percent solution = ppm 10,000

(B) ppm solution = % x 10000 = % x 104

1.1 Example

To prepare a 10 per cent CuS04 solution.

We add 10 g of CuS04 to 100 ml of water. On the other hand, if both the solute and the solvent are liquids, per cent solution is prepared on volume basis, and here, a known volume of the solute is taken and the final volume is then made up to 100 ml with the solvent.

1.2 Example

To make a 35 per cent solution of perchloric acid.

35 ml of perchloric acid is taken in a measuring cylinder and the volume is made up to 100 ml with water. This method of expression does not show the relative number of molecules of the solute that are present in the solution. Therefore, although per cent solutions are used quite frequently in the laboratory, they are generally found inadequate for precise work. When we use salts while preparing a per cent solution, based on weight by volume, it will give per cent of salts but not different ions in the salts.

1.3 Example

To prepare percentage solution of an ion present in the salt (for e.g., Na in NaOH).

The ratio of the ion to the total salt has to be determined. This is calculated based on the molecular weight of the salt to the molecular weight of the ion.

Mol. wt. of NaOH = 40

Na = 23, 0 = 16, H = 1

23 g of Na is present in 40 g of NaOH.

To prepare 10 per cent Na solution, 10 g of Na has to be dissolved in 100 ml H20.

23 g Na is present in 40 g NaOH. 17.4 g of NaOH contains 10 g of Na.

2. Normal solutions

A normal solution contains 1 g equivalent weight of a dissolved substance (solute) per liter of the solution. 1 g equivalent wt. 1 g mol. wt. of the substance of a substance = No. of replaceable hydrogen atoms or hydrogen equivalents Hydrochloric acid (HC1) with mol. wt. of 36.47 has one replaceable hydrogen atom, hence 1 liter of 1 N HC1 contains 36.47 g of the acid. On the other hand, sulfuric acid (H2S04) having a mol. wt. of 98, has 2 replaceable hydrogen atoms. Therefore a normal solution of $H2S0_4$ contains 49g (98/2) of the acid per liter. Similarly 1 N NaOH will contain 40 g of the base per liter (as one hydrogen ion is equivalent to one acid hydrogen atom), while 1 N Ca(0H)2 contains 74.096/2 = 37.048 g/liter. Salts are also considered in the same terms. 1 N K_2SO_4 contains 174.26/2 = 87.13 g/liter (K2S04 contains 2 hydrogen equivalents).

2.1 Example

To prepare 1 N NaOH, dissolve 1 g eq. wt. (40 x 1 = 40) of NaOH in a small quantity of water and make up the volume of 1 liter. To make 2N NaOH, dissolve 40 x 2 g (80 g) NaOH in a small quantity of water and make up the volume to 1 liter. To make 0.1 N NaOH, dissolve 40 x 0.1 g (4.0 g) of NaOH in a small quantity of water and make up the volume of 1 liter.

3. Molar solutions

A molar solution is one containing as many grams of the solute per liter of the solution as the molecular weight of the dissolved substance, i.e., one gram molecular weight of a substance dissolved in 1 liter of the solution.

When we have to prepare the molar concentration of an ion in a substance the molecular ratio of that ion to the total molecular weight of the compound has to be considered.

3.1 Example

NaOH - Mol. wt. = 40

40 g in a liter = 1 M NaOH

To get 1 M of Na the amount of Na needed is 23 g which is present in 40 g of NaOH.

3.2. Example

K2S04 - Mol. wt. =174.2

174.2 g in a liter = 1 M K2SO4

To get 1 M of K the amount of K2S04 needed is 87.1 g (174.2/2 = 87.1)

4. Parts per million (ppm)

One mg of a substance dissolved in 1 liter of a solution will give 1 ppm solution. The quantity of substances necessary to prepare 10 ppm solution is same for substances differing in molecular weight.

4.1 Example

GA - mol wt. = 342.0

10 mg of GA in a liter - 10 ppm

IAA - mol wt. = 175.

10 mg of IAA in a liter = 10 ppm

- Though the molecular weight is different, the amount necessary to prepare 10 ppm is 10 mg. Following formula is used for conversion of hormonal strength.

  ppm solution = % x 10000

  = % x 104

Preparation of hormonal powder:

- For preparation of hormonal powder, the required quantity of hormone is weighed precisely with the help of sensitive balance.
- It is dissolved in ½ litre ethanol, methanol or acetone in a beaker. This material is poured into one kilogram of talc taken in mortar and mixed thoroughly with a glass rod.
- After mixing, the mixture is kept open in air for few hours. The alcohol will evaporate soon, after which, the dried talc is ground to a fine powder (Fig. 6.1).
- This fine powder should be kept in air tight containers to avoid moistening and can be used as and when required.

Figure 6.1 Cuttings treated by hormonal powder

Preparation of hormonal paste:

- For preparing hormonal pastes, the required quantity of the hormone is weighed accurately and dissolved completely in a few drops of alcohol.
- The required quantity of lanolin (wool fat, a product similar to grease and is greenish-yellow in colour) is weighed and heated slightly in a beaker under gentle flame.
- When the lanolin is slightly liquefied the dissolved hormone is poured in it.
- The mixture is dissolved thoroughly with constant stirring with a glass rod. The mixture is allowed to cool down.
- The paste is ready for use. Until use, the paste may be kept for few months in a cool dry place but one should prefer to use fresh paste.

Precautions:

- First of all check the expiry date of the hormone powder.
- The weight should be taken precisely, preferably on electronic balance.
- Proper solvent should be used to avoid precipitation.
- Hormones deteriorate under high temperature, so store in cool and dry place. Hormones are photosensitive; therefore they must be stored in dark or amber colored bottles.
- Use hormonal solutions for treatment of cuttings and lanolin paste for layers.
- Solutions should be prepared fresh. If required to store for some time use, refrigerators.
- The treated cuttings should be planted with the help of some stick to make hole, so as to avoid removal of solution from basal end of cutting.

Methods of application of growth regulators:

The effectiveness of growth regulators not only depend the concentration, but also on the method of application. Auxins are most effectively and widely used rooting hormone. Among synthetic auxins IBA and NAA are found to be most effective for inducing rooting.

The different methods used for treatment of cuttings and layers are as under:

(a) Prolonged soaking method:

- In this method, the basal end of cuttings is dipped in the dilute solution of the hormone for 24 hours in a cool dry place.
- The concentration of hormone or growth regulator usually varies from 20ppm to 200 ppm, depending upon plant species and type of cutting.
- After treatment cuttings are planted in growing medium. The concentration is usually low in growing medium.
- The concentration is usually low for easy to root species and vice versa.
- This method is very useful for difficult to root species, where some materials like vitamins, sugars and nitrogenous compounds are also used along with the growth regulators for facilitating rooting.

(b) Quick dip method:

- In this method, the basal end of cuttings is dipped in the concentrated solution of a hormone for a short time, usually for 5 seconds to 2 minutes depending upon the species to be propagated.
- Treated cuttings are planted in the rooting medium or field. The concentration of hormone for quick dip method may vary from 500 to 10,000 ppm depending upon the type of cutting and species, but generally a concentration of 3000 to 5000 ppm is used.

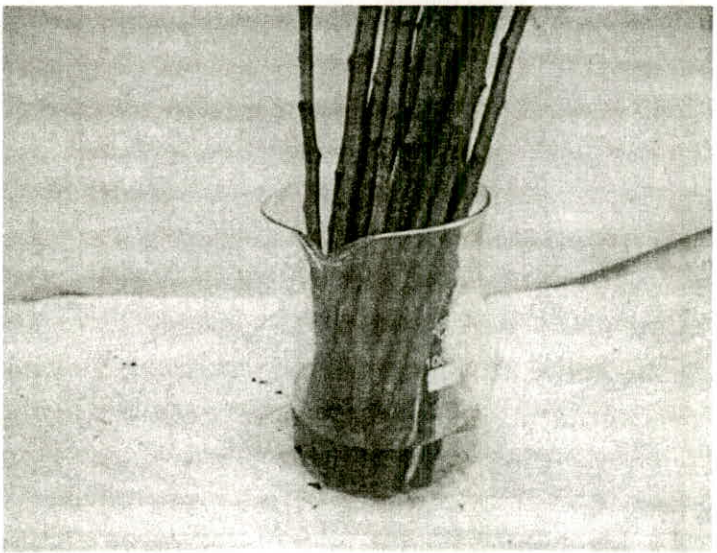


Figure 6.2 Cuttings dippeded in hormonal solution

(C) Powders dip method:

- In this method also basal ends of cuttings are dipped in the hormonal powder which carries (talc) for some time.
- After treatment of cuttings, extra amount of powder adhering to the cuttings should be removed by shaking and cuttings are immediately inserted into the rooting medium.
- For effective rooting, the cut ends of the cuttings should be moistened before the treatment and care should be taken that extra powder adhered to cuttings should be shaken off, otherwise, it may cause adverse effect on the rooting process.
- Seradix, Rootex or many other formulations are available in the market as powders.

(D) Lanolin paste method:

- As described under preparation of hormonal paste, the paste of growth regulators made in lanolin is applied to the girdled portion of a layer or stool for inducing rooting in them.

(E) Spray method:

- Spraying of growth regulators is sometimes done to mother plants before taking cuttings from them. Spraying of stock plants with 2,4,5-T in concentrations ranging from 25 to 100 ppm is done about 30 to 40 days before taking cuttings from them, Cuttings taken from such plants root better as compared to untreated plants.

Growth regulators in excess may inhibit bud development and may cause yellowing and dropping of leaves, blackening of the stem and eventual death of the cuttings. The concentration just below the toxic point is considered the most favourable for rooting media.

Fruit tree	Treated part	Growth substances and their strength	Remarks
Anab-e-Shabi grapes	Hardwood cuttings	IAA (75 mg/l) in leaf mould IAA (100 mg/l) in soil	Better performance on leaf mould
Kagzi lime	Cuttings	IAA (250-300 mg/l) 24 hr dip	Rooting percentage increased
Lemon	Cuttings	NAA (0.2%) percentage	Higher rooting
Sweet lime	Hard wood cuttings	IBA (200 mg/l) with 2% sucrose	Beneficial effect on rooting
Sour orange	Cuttings	IAA and NAA 50 and 100 mg/l	Better rooting
Plum	Stem cuttings	IBA	Recommended for commercial propagation
Citrus (C.aurantifolla)	Air-layers	IBA (1.0%) and IAA (1.0%)	Increased rooting
Guava	Air-layers	IBA (250mg/l in lanolin)	Significant improvement in the rooting

Exercise 7: Identification of physiological and nutritional disorders in fruit crops.

The productivity as well as the quality of fruit crops is affected to a greater extent due to the physiological and nutritional disorders. Disturbance in the plant metabolic activities resulting from an excess or deficit of environmental variables like temperature, light, aeration and nutritional imbalances result in crop disorders. In fruit crops, the deficiency of micronutrients causes many more disorders than that of macronutrients.

Nutritional disorders have become widespread with diminishing use of organic manures, adoption of high density planting, disease and salt tolerance, unbalanced NPK fertilizer application and extension of horticulture to marginal lands. To get high quality fruit and yields, micronutrients deficiencies have to be detected before visual symptoms are expressed.The deficiencies of Zn, Mn and B are common in sweet orange, acid lime, banana, guava and papaya in India. To correct both visual and hidden micronutrient deficiencies, appropriate foliar and soil applications are necessary. The description of physiological and nutritional disorders in crops includes a number of technical terms and it is essential to understand the terms for better identification of symptoms.

Some common terms of disorders :

Bronzing means development of bronze or copper colour on the tissue.

chlorosis means loss of chlorophyll resulting in loss of green colour leading to pale yellow tissues.

Decline means onset of general weakness as indicated by loss of vigour, poor growth and low productivity.

Die-back means collapse of the growing tip affecting the younger.

Leave firing means burning of tissue accompanied with dark brown or reddish brown colour.

Lesion means a localized wound of the leaf/stem tissue accompanied with loss of normal Colour.

Necrosis means death of tissue.

Scorching means burning of the tissue accompanied with light brown colour resulting from faculty spray, salt injury, etc.

The principal causes for the onset of physiological maladies are found to be sudden shift in the environments factors, change in the fertilizer pattern, quality of irrigation water, pest infestation, misuse of chemicals such as herbicides, growth regulators, pesticides, etc.

Mango

Physiological disorders

1. Malformation

There are two types:

(A) Vegetative Malformation

The numerous small shootlets, arising from apex of shoot or leaf axil, thickened and clustered together, giving the twig a broom like appearance commonly known as 'Bunchy Top'.Trees between 4 to 8 years age suffer the most (90.9 %) from vegetative malformation. The axillary buds get swollen and persist on mother tree for a longer time as dry masses.

(B) Floral Malformation

This is an abnormal development of inflorescence in which individual flowers in a panicle get

greatly enlarged with large discs and the peduncle bearing the flower is also much thickened. The affected panicles continue to bear flower buds even after the fruit setting in the normal panicles is over. The malformed flowers remain green for longer period and hang on the trees even after the fruiting season. The percentage of hermaphrodite flowers in a malformed panicle is very low and, therefore, such panicles rarely bear the fruits and if fruit setting takes place, at all, the fruits drop down at the pea stage size. The malformed panicles may be loose or compact.

Causes of Malformation

- The causes of mango malformation are still a matter of controversy.
- There are a number of schools of thoughts and accordingly virus, fungus, mites, nutrients, role of C/N ratio.
- Carbohydrates, nucleic acids, amino acids, proteins, phenolic compounds, enzymatic activity in plants, phytohormones and occurrence of malformin like substances etc. are reported to play major role in the occurrence of the malady.

Recommendations

- Spray of NAA (100 to 200 ppm) during the first week of October has been worked out to reduce floral malformation.
- Deblossoming at the bud burst stage alone and in combination with the spraying of 200 ppm NAA was reported very effective.
- The delayed panicle malformation and induced healthy panicles were observed with spraying of glutathione (560 ppm), AgNO3 (2400 ppm), ascorbic acid (1055 ppm) and K2S2O5 (560 ppm).
- Soil application of N:P2O5:K2O (9:3:3) causes high N and low P and K reduced floral malformation.
- Pruning of Malformed Parts
- The application of 0.04% diazinon controls malformation.
- Polythylene film covers are used around mango shoot in winter. which controlled malformation.

(2) Spongy Tissue :

It is a major problem in Alphonso, where a pulp patch fails to ripen. This malady is caused due toinactivity of ripening enzymes due to high temperature, convective heat and post harvest exposure to sunlight.

Recommendations

- Use of mulching and post harvest exposure to low temperatures between 10-15^0 C for 10-18 hours has been useful in reducing the malady.

(3) Biennial Bearing :

The term biennial, alternate or irregular bearing generally signifies the tendency of mango trees to bear a heavy crop in one year (On year) and very little or no crop in the succeeding year (Off year). Most of the commercial varieties of north India, namely, Dashehari, Langra and Chausa are biennial bearers,while south Indian varieties like Totapuri Red Small, Bangalora, and Neelum are known to be regularbearers. When a tree produces heavy crop in one season, it gets exhausted nutritionally and is unable to put forth new flush thereby failing to yield in the following season. The problem has been attributed to the causes like genetical, physiological, environmental and nutritional factors.

Recommendations:

- For overcoming biennial bearing, deblossoming is recommended to reduce the crop load in the 'On' year so that it is balanced in the 'Off year.
- Proper maintenance of orchard by way of effectivecontrol of pests and diseases and regular cultural operations may also result in better performance of the tree every year.
- Soil application of Paclobutrazol (PP) or Cultar @ 4 g/tree in the month of September resulted in 333 early flowering with higher fruit set and yield. It may be applied every year for regular fruiting, particularly in young trees.
- Ringing of branches is recommended as means of inducing flowering in the 'Off' year.

(4) Fruit Drop :

The intensity of fruit drop varies from variety to variety. Among the commercially grown varieties, Langra is more susceptible to drop, while Dasheri is the least. The fruit drop is more or less a continuous process and can be classified into three phases, viz.

(i) Pinhead drop (ii) Post-setting drop (iii) May-month drop.

The fruit drop in first two phases are insignificant compared to the third phase which affects the final yield significantly and needs more attention. Embryo abortion, climatic factors,disturbed water relation, lack of nutrition, attack of disease and pest and hormonal imbalances are the major factors that lead to fruit drop.

Recommendations:

The foliar application of Alar (B-nine) @ 100 ppm or NAA 20 ppm at pea stage of fruit was found effective in controlling fruit drop in mango.

(5) Black tip

Coal fumes of brick kilns containing sulphur dioxide, ethylene and carbon monoxide are observed to be responsible for black tip. The damage has been noticed in the mango orchards located upto 200 metres of distance from brick kiln. It is characterized by depressed spots of yellowing tissues at the distal end of the fruit, which gradually increase in size, become brown and finally black. The necrotic area is always restricted to the tip of the fruit. The growth of the fruit is almost at stand still and the fruit becomes soft after premature ripening. Such fruits never reach full maturity and drop earlier.

Recommendations:

- Orchards 1.5 km to the east and west and 0.75 km to the north and south away from the kilns.
- Spraying of 2% sodium carbonate or 0.6% borax is recommended as control measure.

Banana

(A) Physiological disorders:

1. Choke throat

It is due to low temperature affecting active growth of the plant. Leaves become yellow and in severe cases, the tissue gets killed. In case of normally flowering plants, the stalk carrying bunches elongates freely so that the entire inflorescence comes out of the pseudostem and hags down. Bunch development is normal, but when the time of flowering synchronizes with low temperature, the bunch is unable to emerge from the

pseudostem properly. The distal part of the inflorescence comes out and the basal part gets stuck up at the throat. Hence, it is called **Choke throat**. Maturity of the bunch is delayed by taking 5-6 months instead of 3.5 - 4 months for harvest.

Recommendations:

Precautions provision of shelter belts, planting low temperature tolerant varieties adjusting the time of planting and orchard to prevent rapid cooling during cold spells.

2. Maturity bronzing

It is a stress related disorder commonly called as maturity stain or bronzing. The symptoms include cracking of the peel and discolouration begins as a light brown tinge and turns to solid dark brown lesions which dries and form longitudinal cracks. The cells of the underlying layers of banana peel expand at a faster rate for a long period of time than epidermal cells. This pattern of growth gives rise to point of weakness at the cell edge. The epidermal cells are subjected to circumferential stress during fruit growth and it exceeds its elastic limit and separation of cells at anticlinal walls of adjacent epidermal cells. Hence, red brown discolouration occurs.

3. Chilling injury

Chilling occurs when pre-harvest or post-harvest temperatures fall below 14°C for various time periods (Stover, 1972). The peel of banana become dark and the fruit exhibit uneven ripening. Ripening fingers show dull yellow to smoky yellow colour and watery dark patches are observed on the skin. Brittleness of the fruit and fungal invasion is also observed. The vascular bundles of the sub-epidermal layer show brown streaks. The discoloration is ascribed to the enzymatic oxidation of dihyroxy phenylalanine.

4. Finger drop

Finger drop is related to the reduction in the pedicel rupture force of fruit. Fruits which were ripened at 15-30°C had progressively weaker pedicels at increasingly high temperature. However, at very high temperature of 40°C pedicels remained firm and fruits were resistant to finger drop. During ripening, the peel of banana loses weight more rapidly than pulp and becomes easy to peel. Fruits ripened at 40°C had a very high weight loss which resulted in thin dry leathery skin which is resistant to finger drop. Exposure to ethylene for 24 hours reduce finger drop because of a faster ripening rate and high water loss.

5. Hard lump

Hard lump or masses of varying sizes and shapes occur in the pulp. Lumps are pinkish brown in colour and more firm than the usual pulp and taste like immature or unripe fruits, with the result the quality of the fruit is affected. The pink discoloration and astringent taste of fruit is due to high accumulation of tannin during winter months which inhibit the conversion of starch to sugar. Spraying or dipping the end of peduncle in 20 ml of 2, 4-D 1000 ppm checks the disorder.

(B)Nutritional disorders

Lopex and Espinosa (2000) observed that banana required more nutrients per hectare than any other commercially cultivated crop and reported various nutritional disorders affecting the yield and quality of banana.

1. Nitrogen deficiency

The most evident symptom that N is lacking is yellow leaves resulting from a reduction in chlorophyll content. The normal progression of the deficiency is for yellowing to begin in the older, lower

leaves and subsequently to affect younger leaves as the deficiency worsens. Petioles of the most affected leaves show a pinkish discoloration.

2. Potassium deficiency

The most characteristic of the K deficiency symptoms is the yellowing of older leaf tips followed by inward leaf curling and death. The banana bunches in K deficient plants are short, slim and deformed as a consequence of poor fruit filling caused by reduced photosynthesis and sugar transportation.

3. Calcium deficiency

Calcium deficiency symptoms appear in the younger leaves since it has low mobility within the plant. This thickening is accompanied by marginal interveinal chlorosis as the plant grows. If the deficiency is severe, the leaves become completely deformed with margins becoming serrated or saw-like. Prolonged and severe Ca deficiency can prevent the emergence of new leaves and ultimately the death of the plant.

4. Magnesium deficiency

The most typical visual Mg deficiency is yellowing of the outside section of the lamina in older leaves, which is due to its mobility in the plant. As the deficient leaf matures, chlorosis becomes more intense, and dark spots form, that later become necrotic. Affected leaves become an intense golden yellow colour. The deficiency changes the arrangement of leaves in the pseudostem, producing a rosette appearance.

5. Zinc deficiency

The most typical visual Zn deficiency is the leaf width reduced more than the length and the leaf becomes lanceolate in shape. The lower leaf surface shows purple pigmentation, resulting in interveinal chlorosis and yield reduction. Spraying of Zinc sulphate 0.3% + 0.5% urea at 45 and 60 days after planting of main crop and 45 days after cutting of mother plant for ratoon crop corrects the disorder well.In Zn deficient soils, application of Zinc sulphate @ 50 g/plant at the time of planting is recommended.

6. Boron deficiency

In B deficient plants, the veins are very close, raised above the lamina and leaves are brittle in the early stage. In the later stage, chlorotic spots parallel to midrib and corrugation and laddering symptoms also appear and unfolding of leaf is delayed in addition to the yield reduction. Breading of leaftip, torning of leaf tips and browning at the end is also observed. Corrective measure is application of borax at 20 g/plant at the time of planting and foliar spray of 0.2% boric acid at fourth and fifth month of planting.

Acid lime

(1) Iron chlorosis

Iron deficiency is known simply as 'Iron chlorosis or as lime induced chlorosis'. It develops on young growth but may persist throughout the life of the plant. In mild cases young leaves become pale green except for the veins which remain dark green. In severe cases, the symptoms are drastically aggravated. Newly emerging leaves are almost white with only a faint tinge of green along their midribs. The tree may bear fewer fruits which are small, hard and coarse.

Recommendations

- 500 gm of ferrous sulphate and 100 gm of urea along with 100 ml of soap solution are dissolved in 100 litres of water and sprayed to the crop in an are of garden.
- The spray may be repeated two or three times at an interval of 20 days depending on the extent of deficiency.

Sathukudi

(1.) Little leaf with chlorosis

The leaves are very much reduced in size and will exhibit chlorotic symptoms.

Recommendations

- Foliar spray of manganese sulphate and zinc sulphate each 500 gm along with 100 gm of urea dissolved in 100 litres of water is done three limes at 20 days interval.
- soil application of manganese sulphate (100 gm) and zinc sulphate (100 g) along with 25 kg of compost farm yard manure will be advantageous.

Sapota

Boron Deficiency

When there is boron deficiency, the pollination of flowers is affected leading to sterility; hence, fruit formation is affected resulting in poor yield.

Recommendations

- Foliar spray of boron (300 gm) / 100 litres of water at the time of flowering is to given twice at 15 days interval.
- Boron nutrition will help in proper pollination and fertilization of flowers in sapota.

Guava

Bronzing of leaves

The leaf size will get reduced and leaf colour will turn yellowish or reddish brown. Fruit size also will be reduced with surface cracking and malformation.

Recommendations

- 300 g each of borax, zinc sulphate and copper sulphate along with 100 g of urea and 100 ml of soap solution dissolved in 100 lit of water will be sufficient for an area of one acre of garden.
- Basal soil application of 30 g each of borax, zinc sulphate and copper sulphate has to be done around each tree. These fertilizers are mixed with compost and then placed in the trenches around the trunk of the tree; it is to be followed by immediate irrigation.

Grapes

Hen and chicken disorder

This disorder is characterized by the presence of a large number of shot berries along with a few normal size berries. The leaves will be marketed by presence of yellow spots and wilting of leaf tip and margins. The fruits will be sour to taste.

Recommendations

Since this disorder is caused mainly by boron deficiency, foliar application of borax @ 500 g along with 100 g of urea and 100 ml of soap solution in 100 lit of water is to be given for an orchard of one acre area.

Exercise 8: Identification of nutritional disorders in vegetable crops.

What is deficiency symptom?.

When nutrient is not present in sufficient quantity, plant growth is affected. Plants may not show visual symptoms up to a certain level of nutrient content, but growth is affected and this situation is known as hidden hunger. When a nutrient level still falls, plants show characteristic symptoms of deficiency. These symptoms, through vary with crop, have a general pattern. These are generally masked by diseases and other stresses and so need careful and patient observation on more number of plants for typical symptoms. The deficiency symptoms appear clearly in crops with larger leaves.

Identification of Deficiency Symptoms:

The deficiency symptoms can be distinguished based on the (1) region of occurrence, (2) presence or absence of dead spots, and (3) chlorosis of entire leaf or intervenial chlorosis.

In most of the vegetable crops some common deficiency symptoms of macro, secondary and micronutrients appear in different parts of plants which depends upon the type of crops and nutrients. The major deficiency symptoms which occur in vegetable crops are given below:.

Nitrogen: Stunted growth, young yellowish green leaves and older leaves light green followed by yellowing and drying or shedding. Reduced fruit size.

Phosphorus: Young plants stunted, leaves dark blue-green (or) reddish purple colour, stems slender. Arrest in meristematic growth. Delay in maturity of fruits.

Potassium: Slow growth of plant Curling, bronzing and drying of margins. Brown spots throughout, uneven fruit ripening.

Calcium: Weak stem and slow growth. Leaves chlorotic with necrotic spots. Tips of young leaves curl backward margins waved and irregular. Little or no fruiting.

Magnesium: Mottled yellowing (Veins green and leaf web tissue yellow or white) on older leaves. Necrosis (Brown spots) on leaves. Delay in maturity.

Sulphur: Stems often slender. Yellowing along the veins of young leaves.

Iron: Interveinal chlorosis of young leaves. Sometimes leaves are completely bleanched, margins and tips scorched.

Zinc: Young leaves growth affected - Rosetting. White chloritic streaks between veins in older leaves. Leaves chlorotic and necrotic in younger leaves. Whitening of upper leaves in monocots, chlorosis of lower leaves in dicots.

Manganese: Mottled chlorosis (veins green and leaf web tissue yellow or white), appearing first on young leaves. Little fruit formation.

Copper: Wilting of terminal shoots followed by death. Yellowing of leaves in lettuce.

Boron: Plants dwarfed or stunted. Yellowing or browning of leaf margins. Curling of younger leaves. Flower development or seed production normally impaired.

Molybdenum: Light yellow chlorosis of leaves.

Visual nutritional deficiency symptoms of different vegetable crops:

1. Cabbage

Visual symptoms include mottling, scorching, wilting and frequent cupping of older and middle leaves malformation or death of the growing point also occur (Hewit and Bolle-Jones, 1952). In cabbage tip burn appears as necrotic spots or areas in the margins of the rapidly expanding leaves in the middle part of the head.

2. Cauliflower

Nitrogen: Causes marked reduction in growth. Leaves small in size young leaves are pole green in colour, while old leaves turn purple. The curd formation is delayed Mehtrotra and Mishra(1974).

Calcium: Physiological disorders related to lack of calcium in the affected organ are common in the cole crops. Necrosis of the edges of young, rapidly expanding leaves is characterized by tip burn disorder. Tip burn of cauliflower also appears in the margin of immature leaves near, the developing curd (Rosen, 1999) and the curd may be discoloured if the dead leaf tissue touches it. In green house and growth chambers result in more severe calcium deficiency disorder, the production of translucent or "glassy" curds.

Boron: Mehrotra and Misra (1974) observed stiff Stem with hollow core, curled leaves, leathery due to born deficiency. Delay in curd formation which turns to dirty pale to brown in colour.

Zinc: Leaf number of size reduced. Leaves become mottled and necrotic (Mehrotra and Misra, 1974).

Copper: Under copper deficient conditions according to Mehrotra and Misra (1974) the leaves become small and bluish in colour.

Iron: Interveinal chlorosis of young leaves and latter wholly bleached.

Manganese: Wild gray interveinal mottling and necrosis of older leaves. Young leaves remain normal, but turn chlorotic between veins and spread gradually to older leaves.

Molybdenum: Blindness is the loss of growing point and is reported to be associated with: low temperature, molybdenum deficiency, which causes whiptail disorder. Cauliflower requires high molybdenum. It is an indicators crop for this nutrient (Falkl and Podleasak, 1983). Young plants show chlorotic and may turn white along the leaf margins, also become cupped and wither. In older plants, the lamina of the newly formed leaves are irregular in shape, frequently consisting of only a large midrib and hence the name whiptail. At low soil pH of 4.6, a mixed syndrome of Mo deficiency and Mn toxicity appear on the same plant (Plant, 1956).

3. Brussels sprouts

Calcium: In this crop the calcium deficiency disorder is turned as internal browning (Millikan and Hanger, 1966). Several calcium deficiency in this also occurs as a marginal necrosis of the young leaves near the shoot.

Manganese: This crop is highly sensitive to Mn toxicity. Leaves show chlorotic and crop stunted.

Molybdenum: Interveinal chlorosis, stunted and straggely older leaves drop off. Sometime 'whiptail' disorder is also seen.

4. Broccoli

In India it is hardly considered as a commercial crop. Hollow stem of broccoli is related to higher dose of nitrogen.

Sulphur: Severe stunted growth, leaf blade become thickened.

Molybdenum: Whiptail disorder.

5. Carrot

The cavity spot in carrot, a typical calcium deficiency disorder caused by high potassium application especially under water logged conditions, where ammonium is mainly responsible for reduced calcium uptake (Dekock et al., 1981) and also related to weather. Physiological disorders of carrot

(a) Forking: Damage to the tap root of carrot seedlings cause forking (i.e. splitting).

(b) Damage and cracking: Splitting (longitudinal cracking) and transverse breakage which occurs after harvest.

6. Radish

Nitrogen: Marked reduction in growth: Size and number of leaves are reduced. Pale green colour of leaves turn to yellow. Roots are their, stiff and fibrous. (Roy Choudhury et al., 1982).

Phosphorus: Stunted plant growth. Small leaves, distorted in shape, pink tinge colour along the leaf margins and veins.

Potassium: Colour of leaves change from green to pale yellow, brown scorches appear on the leaves at later stages violet streaks appear on root.

Calcium: Fewer leaves with small roots in size. Chlorosis of young leaves.

Magnesium: Roy Choudhury et al. (1982) reported that magnesium deficient plants show chlorosis on mature leaves which abscissic later. The roots are small in size, stiff and pale in colour.

Sulphur: Yellowing of margin of young leaves and spread towards inside.

Iron: Chlorosis of young leaves. Storage roots are reduced in size and become pale in colour.

Lead: The toxicity symptoms lead include wilting, chlorosis, necrosis, and banding of roots.

7. Pea

Zinc: Leaes are narrow, pointed and curled in wards. Yellow mottling on the lamina started from the base of the midrib and move upwards. Poor pod set and seed development.

Iron: Chlorosis of young leaves.

Copper: Thin and weak stem bend upward. Lower leaves are narrow and pointed but upper ones are broad and large. Older leaves yellow and shed. Poor fruit set.

Manganese: Marsh spots (Minute brown spots) on the older leaves during flowering which become yellow mottled in the interveinal areas. Flowers shed after fading and seed development is affected.

Boron: Stunted growth with small and thick leaves followed by death of the apical growing point results in development of lateral branches. Chlorosis of margins of leaves, later turn to dirty yellow and roll inward. Delayed flowering, shedding of flower poor fruit / pod set.

Molybdenum: Mottling of leaves accompanied by death of most of the interveinal leaf tissue (Meagher et

al., 1952). Yellowing of older leaves and curving upwards from the margins resulting in cup shaped appearance also occur. Flowering and fruiting reduced considerably.

8. Spinach

Nitrogen: Restricted growth, leaves are stiff and small in size, yellowing delayed flowering.
Phosphorus: Stunted growth and leaves become dark green in colour.
Potassium: Fewer small leaves, stunted growth, leaves pale yellow, dry spots on the leaf lamina.
Magnesium: Chlorosis of mature leaf, reduced leaf number.
Calcium: Chlorosis of leaves at early stage of development.
Iron: Yellowing of leaves, leaf size small, interveinal chlorosis of young leaves.

9. Celery

(1) Blach heart disorder: This physiological disorder is mainly due to calcium deficiency. Burning of tip of the young developing leaves become necrotic, first at the tip then spread all over the leaf. In severe cases, all the interior leaves (Heart) can become rotten (Geraldson, 1954).
(2) Petiole pithiness: Petiole pithiness is a widespread disorder in stalk celery, the petiole of the crop are naturally hollow due to the breakdown of parenchyma cells. Leaving larger spaces in the parenchyma which under severe conditions may merge with one another and form a hollow petiole.
(3) Brown cracking: Brown cracking caused due to boron deficiency.

Exercise 9: Diagnosis of deficiencies through rapid tissue testing

Plant tissue analysis could directly reflect the nutrient status or nutrient requirement of plants themselves. In recent years probably as a result of advances in knowledge and understanding of the role and function of nutrient elements, new approaches to diagnosis are being developed which differ in principle from plant analytical techniques. These are based on specific physiological or biochemical changes caused by deficiencies or alternatively, on specific responses that can be induced in plants or plant tissue by the addition of a deficient element. There are two types of plant analysis for confirmation of different symptoms and also for assessing the nutrient status at particular stage of the plant.

There are two types of plant analysis

1. Tissue testing
2. Whole plant analysis

Tissue testing is done usually with fresh leaves of the plant in the field itself whereas the total plant analysis is performed in the laboratory. These plant analysis methods are based on the assumptions that the particular element is an indicator of the supply of that particular nutrient. The whole plant analysis methods involve elaborate equipment and a lot of chemicals and cannot be performed in the field itself. However, tissue testing is done in the field itself and also very rapid. The test is made with fresh plant saps and very useful in quick diagnosis of the needs of growing plant. In this test, the sap from the cell is tested for unassimilated N and K. Test for Fe, Ca and Mg is also used frequently in variety of crops. In general, it is necessary to test that specified part of the plant, which will give the best indication of the nutritional status.

Crop	Nitrogen	Phosphorus	Potassium
Papaya	Petiole	Petiole	Petiole
Tomato	Petiole	Petiole	Petiole
Banana	Lamina	Lamina	Lamina

(1) Nitrogen

Reagents

0.1% of Diphenylamine in concentrated sulfuric acid.

Procedure

- Small bits of leaf or petiole are taken in a petridish and a drop of 0.1%. diphenylamine is added.
- The development of blue color indicates the presence of nitrate nitrogen.
- Depending on the intensity of blue color the nutritional status may be diagnosed as sufficient or not sufficient.

  (1) Dark blue - Sufficient (2)Light blue - Slightly deficient (3)No color - Highly deficient

(2) Phosphorus

Reagents:

Ammonium molybdate reagent:

- 8 g of ammonium molybdate is dissolved in 200ml of distilled water.
- To this solution, a mixture of 126ml concentrated hydrochloric acid and 74ml of distilled water is added slowly by constant stirring.
- This stock solution is kept in an amber colored bottle and at the time of use, it is diluted in the ratio of 1:4 with distilled water.

Procedure:

- Small fine bits of the plant material are taken in a test tube and 10ml of diluted reagent is added and shaken continuously for a minute.
- To this a pinch of stannous chloride powder is added. The contents are mixed thoroughly and observed for color development.

(1) Dark blue - Rich in phosphorus (2) Light blue - Moderately sufficient

(3) Green or bluish green - Deficient (4) No color or yellow - Highly deficient

(3) Potassium, Calcium, Magnesium and Chloride

With Morgan's reagent, the following elements would be detected as soluble potassium, calcium, magnesium and chloride.

Morgan's reagent

10g of sodium acetate is dissolved in 30ml of glacial acetic acid (pH 4.8) and used for the extraction.

Preparation of plant extract

- 4g of plant sample is taken and 15ml of Morgan's reagent is added.
- A pinch of Darco is added and filtered through muslin cloth.
- This extract could be subsequently used for detection.

(a) Potassium

Reagents

35% sodium cobalt nitrite, 50% Glycerin, Isopropyl alcohol

Procedure

- 2ml of Morgan's reagent extract is taken in a test tube and to this 0.2ml of sodium cobalt nitrite, 1ml of 50% glycerin and 2 ml of isopropyl alcohol are added and observed for color development.

(1) Clear reddish brown - Insufficient (2) Deep canary yellow turbidity - Sufficient

(b) CALCIUM

Reagents

50% Glycerin, Ammonium oxalate

Procedure

- To 2ml of Morgan's reagent extract, 2ml of 50% glycerin and 5ml of saturated ammonium oxalate are added.

(1) Colorless - Insufficient (2) Greenish white turbidity - Sufficient

(c) Magnesium

Reagents

0.15% Titan yellow, 2% Hydroxylamine hydrochloride,5% Sucrose,10% Sodium hydroxide

Procedure

- 2ml of Morgan's reagent extract is taken in a test tube.
- To this 2ml of Titan yellow, 0.5ml of hydroxylamine hydrochloride, 0.5ml of sucrose and 2ml of 10% sodium hydroxide are added and observed for color development.

(1) Straw yellow - Insufficient (2) Salmon pink color - Sufficient

(D) Chloride

Reagents

N/50 silver nitrate,Concentrated Nitric acid

Procedure

- To 2ml of Morgan's reagent extract, 2ml of N/50 AgNO3 and 3 drops of concentrated nitric acid are added and kept for color development.

(1) Colorless - Insufficient (2)White turbidity - Sufficient

(E) IRON

Reagents

Concentrated sulfuric acid,Concentrated nitric acid,20% Ammonium thiocyanate in amyl alcohol

Procedure

- 0.5g of the material to be tested is taken in a test tube and 1ml of concentrate sulfuric acid is added and allowed to stand for 15 minutes.
- After that, 10ml of distilled water and 2-3 drops of concentrated nitric acid are added. After 2 minutes, 10ml of this solution is added with 5ml of 20% Ammonium thiocyanate solution and observed for colour development.

(1) Brick red: Sufficient (2) Faint color: Deficient

(F) Manganese

Reagents

Saturated solution of Potassium periodate, 1% tetramethyl diamino diphenyl methane

Procedure

- To finely chopped leaf bits, 2ml of potassium periodate and 0.4ml of 1% tetramethyl diamino diphenyl methane reagent are added.
- The contents are shakenvigorously and observed for color development.

(1) Pale blue - Insufficient (2) Deep blue - Sufficient

Exercise 10: Seed viability by tetrazolium test.

Seed physiology

A true seed is a fertilized mature ovule that possesses an embryonic plant, stored food material and a protective covering (seed coat or testa). The ovule after fertilization consists of embryo sac, zygote and surrounded by nucellus and integuments. In the transformation of an ovule into a seed the integuments become seed coat, thus sealing off the enclosed seed parts from the environment. As a result of cell division the endosperm becomes a multicultural endosperm tissue and serves as a store house of reserve food for the embryo. The zygote develops into embryo. As the embryo increases in size, it absorbs food from the endosperm. The growth of both the endosperm and zygote takes place at the expense of the nucellus, which gradually disappears. If it remains, it becomes a thin layer of cells inside the seed coat. Thus a young seed consists of a protective seed coat, an embryo and stored food, i.e. endosperm which supplies food to the embryonic axis. The endosperm may or may not be present in all seeds. When it is present the seed is called albuminous and when it is absent the seed is exalbuminous (Figure 10.1).

Albuminous Seeds:

Monocots : Rice, Wheat, Maize **Dicots :** Castor, Opium

Exalbuminous Seeds:

Monocots : Orchids **Dicots :** Pea, Bean, Black gram, Green gram

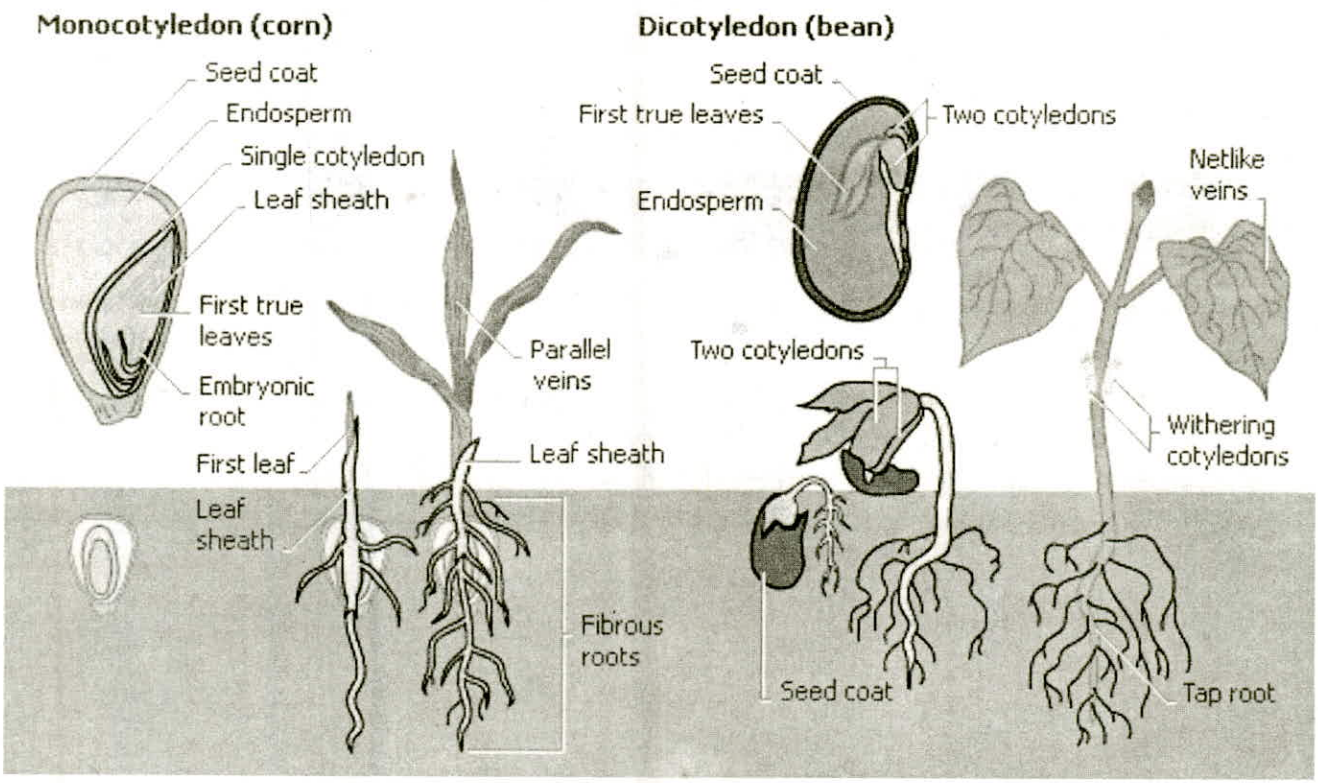
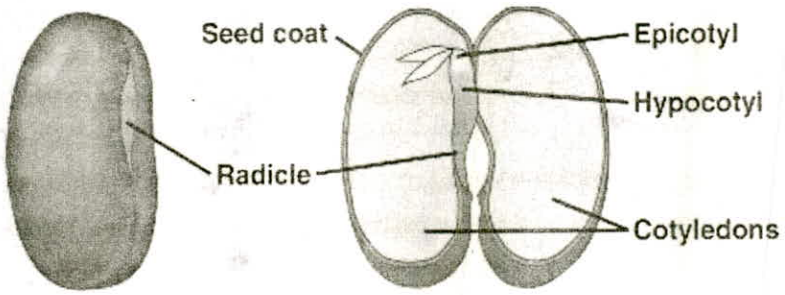


Figure 10.1: Differentiation process of seed to seedling

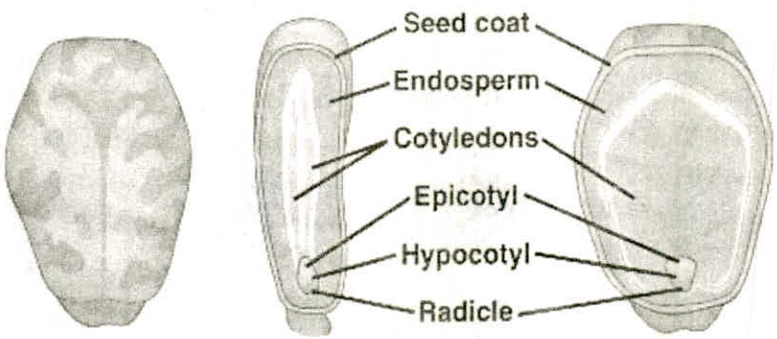
The embryo soon becomes differentiated into a rudimentary shoot or "Plumule", a rudimentary root or "Radicle", the structure connecting the plumule, and radical known as "hypocotyl" and one or more seed leaves called "cotyledons". Plumule, hypocotyls and radical constitute the axis of the seedling; the cotyledons are temporary embryonic structures (Figure 10.2).

Seed viability

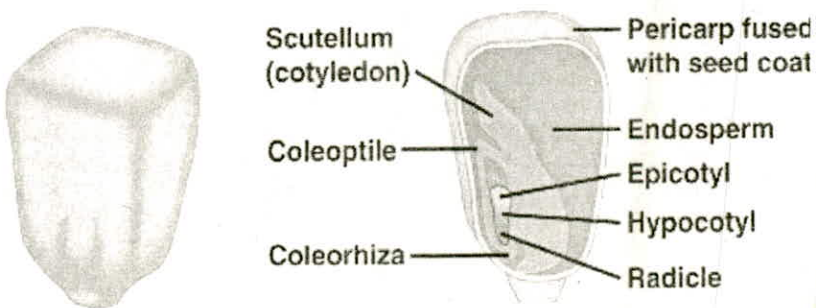
Viability means a seed is capable of germinating and producing a normal seedling. Seed viability test plays an important role in determining the seed quality. The standard practice to test the seed viability is to determine the percentage of seed germination. This, however, is sometimes a very time consuming process. For example, seeds of many plants show post-harvest dormancy, i.e., they germinate only after a certain period of dry storage, which may vary from few days to several months or even years. Numerous tests exist for determining seed viability.



(a) Common garden bean, a eudicot with thick cotyledons



(b) Castor bean, a eudicot with thin cotyledons



(c) Maize, a monocot

Figure 10.2 : Internal embryonic structure of Seeds

Tetrazolium test

Tetrazolium is a simple and quick biochemical method to determine the viability of seeds, which is based on the reduction of a colourless soluble tetrazolium salt to a reddish insoluble substance called formazan in the presence of dehydrogenases. Even in dormant stage, viable seeds show detectable dehydrogenase activity. Therefore, it implies that the seeds when treated with tetrazolium salt turn red are viable and will readily germinate when seeds are provided with necessary environment.

Principle

- The tetrazolium test distinguishes between viable and dead tissues of the embryo on the basis of their relative respiration rate in the hydrated state.
- The highly reduced state of dehydrogenases gives off hydrogen ions to oxidized colourless totrazolium salt solution which is changed into red formazan.

Materials

Dry seeds of Phaseolus, Helianthus, Zea, Ricinus Cucumis etc., 2, 3, 5-Triphenyl tetrazolium chloride solution (1%),Petridishes, filter paper, Incubator

Procedure

- Soak 50 seeds overnight in water. Cut seeds into half by a sharp scalpel, place them in a petri dish with the cut surface in contact with filter paper soaked in sufficient amount of tetrazolium salt and incubate at 20-25°C in the dark.
- Repeat the experiment using seeds which have been previously heated at 100°C for at least 30 min.

Results

Determine after 24 hours the percentage of seeds that have turned red due to the accumulation of formazan. Note that the seeds which were killed by heating will fail to turn red due to the lack of dehydrogenase activity. Although the tissues of living seeds stain red, estimation of viability requires skill and experience. Embryo tissues absorb tetrazolium slowly and tend to develop a lighter colour than embryos that are bruised, aged, frozen or disturbed otherwise.

Methods of seeds preparation for TZ test

Examples:

1. **Corn and Sorghum -** Soak the seed in warm water for 3-4 hours at 30°C, bisect the seed and place in TZ solution.
2. **Groundnut:** Soak the seed overnight in warm water at 20°C-30°C, remove seed coat and use it.
3. **Green gram:** Soak the seed overnight in warm water at 20°C-30°C and use it.

Exercise 11: Physiology of flowering in plants.

What is Photoperiodism?

The plants in order to flower require a certain day length i.e., the relative length of day and night which is called as photoperiod. The response of plants to the photoperiod expressed in the form of flowering is called as photoperiodism.

The phenomenon of photoperiodism was first discovered by Garner and Allard (1920, 22) who observed that the Biloxi variety of Soybeans *(Glycine max)* and 'Maryland. Mammonth variety of tobacco *(Nicotiana tabacum)* could be made to flower only when the daily exposure to the light was reduced below a certain critical duration and after many complex experiments concluded that 'the relative length of the day is a factor of the first importance in the growth and development of plants'. Depending upon the duration of the photoperiod, they classified plants into three categories(Fig. 10.1).

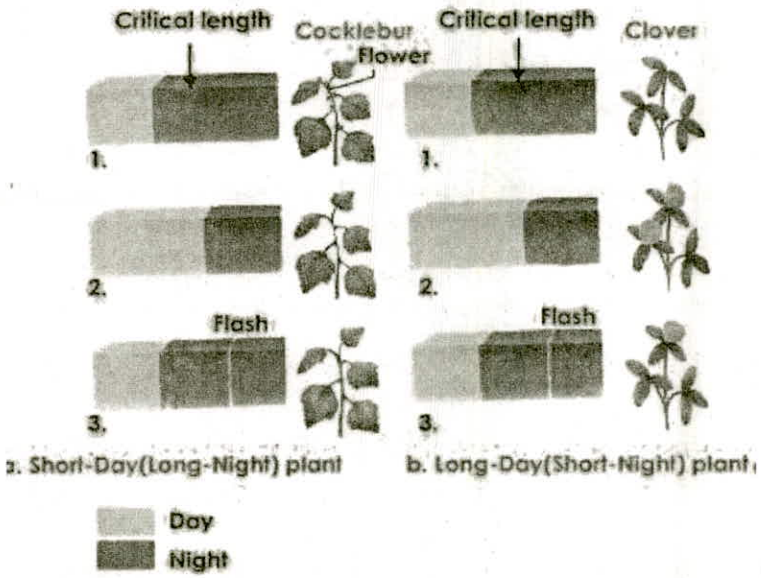


Figure 10.1 the phenomenon of photoperiodism

(1) Short Day Plants (SDP)

These plants require a relatively short day light period (usually 8-10 hours) and a continuous dark period of about 14-16 hours for subsequent flowering. Some examples of these plants which are also known as **long-night-plants** are Maryland Mammoth variety of tobacco *(Nicotiana tabacum)*, Biloxi variety of Soybeans *(Glycine max)* and Cocklebur *(Xanthium pennsylvanicum)*(Fig. 10.1).

(2) Long Day Plants (LDP)

These plants require a longer day light period (usually 14-16 hours) in a 24 hours cycle for subsequent flowering. Some examples of these plants which are also called as short night plants are *Hyoscyamus niger* (Henbane), *Spinacea* (spinach) Beta *vulgaris* (Sugar beet).

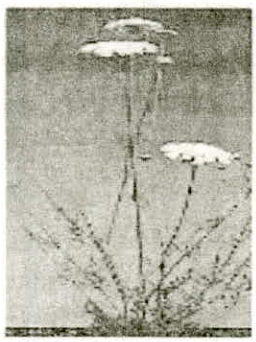
(3) Day Neutral Plants

These plants flower in all photoperiods ranging from 5 hours to 24 hours continuous exposure. Some of the examples of these plants are tomato, cotton, sunflower, cucumber and certain varieties of peas and tobacco. During recent years certain intermediate categories of plants have also been recognized.

VERNILIZATION

What is Vernalization?

Certain plants require a low temperature treatment during their earlier stages of the life history for subsequent flowering in their later stages. This was first realized by Klippart in 1857 when he found that winter wheat could be converted to spring wheat if the seeds after slight germination were kept at nearly freezing temperature (0-5 C). This conversion by low temperature treatment or chilling treatment was termed as vernalization by Lysenko (1928) (Fig. 10.2).


(Fig. 10.2).

Carrot plats (var. Early french forcing). left: control; centre: maintained at 17°c but supplied 10 mg of gibberellin daily for 4 weeks; right: plant given vernalizing cold treatment (6 weeks). All Photographed 8 weeks after completion of cold treatment.

Perception of the cold stimulus

The cold stimulus is perceived by the apical meristems and all dividing cells including those in roots or leaves may be the potential sites of vernalization.

Presence of floral hormone

Perception of cold stimulus results in the formation of a floral hormone which is transmitted to other parts of the plant. In certain cases the cold stimulus may even be transmitted to another plant across a graft union. The hormone has been named vernalin by Melchers (1939).

Conditions necessary for vernilization :

(1) Age of the plant

It determines the responsiveness of the plant to cold stimulus and it differs in different species. In case of biennial variety of henbane *(Hyoscyamus niger)*, the plants will respond only when they are in rosette stage and have completed at least 10 days of growth.

(2) Appropriate low temperature and duration of the exposure

Most suitable temperature is 1-6°C. The effectiveness decreases from 0 to -4°C. Temperature of 4°C is completely ineffective. Similarly from 7°C the response decreases. Temperature 12°C-14°C are almost ineffective in vernalizing the plants.

(3) Oxygen

Vernalizing is an aerobic process and requires metabolic energy. In the absence cold treatment, it becomes completely ineffective.

(4) Water

Sufficient amount of water is also essential. Vernalization in dry seeds is not possible.

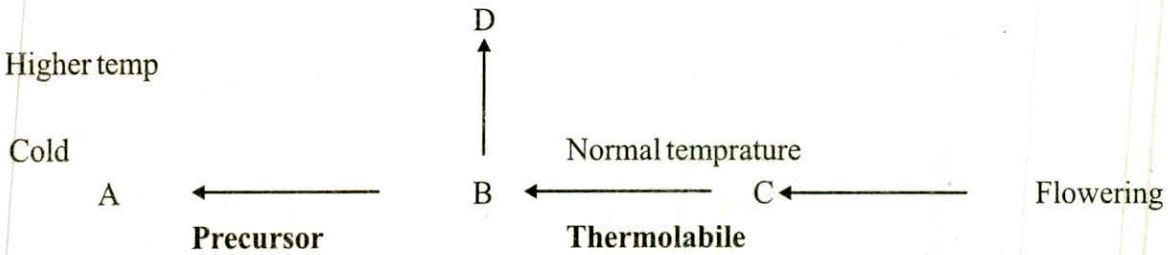
Mechanism of vernilization :

(1) Phasic development theory

The main points in this theory advanced by Lysenko (1934) are the growth and development of an annual seed plant consists of series of phases which must occur in some predetermined sequence. Commencement will take place only when preceding phase is over. The phases require different external conditions for completion like light and temperature. Vernalization accelerates the thermophase.

(2) Hormonal theories

First hormonal theory was proposed by Lang and Melchers (1947) is schematically shown below:



According to the scheme, precursor A is converted into a thermolabile compound B during cold treatment. Under normal conditions, B changes into C which ultimately causes flowering. But at higher temperature B is converted into D and flowering does not take place (devernalization).

Devernalization

The positive effect of the low temperature treatment on the vernaliztaion of the plants can be counteracted by subsequent high temperature treatment. This is called as devernalization. The degree of devernalization decreases if the duration of the cold treatment has been longer. However, the devernalized plant can again be vernalized by subsequent low temperature treatment.

Exercise 12: Seed dormancy and seed germination.

What is seed dormancy?

All the viable seeds have capacity to germinate if placed under suitable conditions necessary for germination. But some seeds fail to germinate for sometimes even if placed under the condition favourable for germination. This may be due to some internal factors or due to specific requirement for some environmental factors. During this period, the growth of the seeds remains suspended and they are said to be in rest stage or dormant stage and this phenomenon is called as dormancy.

Factors causing dormancy of seeds

1. Seed coats impermeable to water

The seeds of certain plants especially those belonging to the families leguminosae, solanceae, malvaceae, etc. have very hard seed coats which are impermeable to water. The seeds remain dormant until the impermeable layer decay by the action of soil micro-organisms.

2. Seeds coats impermeable to oxygen

In many plants such as cocklebur and many grasses, the seed dormancy is due to the impermeability of the seed coat to oxygen. However, during the period of dormancy the seed coat gradually becomes more permeable to oxygen so that they may germinate.

3. Immaturity of the Embryo

In certain orchids, the seed dormancy is due to the immaturity of the embryos which fail to develop fully by the time the seeds are shed. In such cases, the seeds germinate only after a period or rest during which the development of embryo inside the seed is completed.

4. Germination Inhibitors

In certain seeds, the dormancy of the seeds is due to the presence of certain germination inhibitors like coumarin, ferulic acid, abscisic acid, etc. These may be present in endosperm, embryo, testa or juice or pulp of fruit.

5. Chilling or low temperature requirement

In certain plants such as apple, rose, peach etc, the seeds remain dormant after harvest in the autumn, because they have a low temperature or chilling requirement for germination. In nature this requirement is fulfilled by the winter temperatures. In such case, the seeds remain dormant throughout the winter season and germinate only in the following spring.

6. Light sensitive seeds

In many species, the germination of the seeds is affected by light resulting in seed dormancy. Such light sensitive seeds are called photoblastic. Seeds of lettuce, tomato and tobacco - are positively photoblastic and germinate only after they have been exposed to light. On the other hand, the seeds of certain plants are negatively photoblastic and their germination is inhibited by light.

Types of Dormancy

1. Exogenous dormancy

Exogenous dormancy is caused by conditions outside the embryo and is often broken down into three subgroups:

(a) Physical dormancy

Which occurs when seeds are impermeable to water or the exchange of gases.

(b) Mechanical dormancy

Mechanical dormancy occurs when seed coats or other coverings are too hard to allow the embryo to expand during germination.

(c) Chemical dormancy

Includes growth regulators etc. that are present in the coverings around the embryo.

2. Endogenous dormancy

Endogenous dormancy is caused by conditions within the embryo itself and is often broken down into three subgroups.

(a) Physiological dormancy

Physiological dormancy prevents embryo growth and seed germination until chemical changes occur.

(b) Morphological dormancy

Embryo underdeveloped or undifferentiated. Some seeds have fully differentiated embryos that need to grow more before seed germination, or the embryos are not differentiated into different tissues at the time of fruit ripening.

(c) Combined dormancy

Seeds have both morphological and physiological dormancy.

Advantages of dormancy

1. In temperate zones, the dormancy of seeds helps the plants to tide over severe cold which may be injurious for their vegetative and reproductive growth.
2. In tropical regions, the dormancy of seeds resulting from their impermeable seed coats ensures good chances of survival during water stress.
3. Dormancy of seeds in many cereals is of utmost importance to mankind. If these seeds would germinate immediately after harvest in the field, they will become useless to man for consumption as food. Rain, at the time of harvest or maturity may spoil entire produce by initiating germination.

Seed germination

The process of seed germination starts with the imbibition of water by seed coat and emergence of growing root tip of embryo. The optimum conditions for seed germination are availability of moisture, O2 and optimum temperature.

Physiological and biochemical changes during seed germination

Physiological changes

1. Water uptake

Seed germination starts with the imbibition of water by dry seed coat. Due to imbibition of water the seed coat becomes more permeable to O2 and water and less resistant to outward growth of embryo. After imbibition, the inner contents of the seed increase in volume, thereby exerting pressure on the seed coat leading to rupture of the seed coat. The plumule and radical emerge thereafter.

2. Respiration

After initiation of germination process, enormous energy is required for various biochemical changes which are met through rapid increase in respiration rate. Sucrose is probably the respiratory substrate at this stage which is provided by endosperm. In oilseeds and pulses, the lipids and proteins respectively are converted into sucrose by suitable biochemical reactions.

3. Mobilization of reserve materials

As germination progresses, there is mobilization of reserve materials to provide.

i) Building blocks for the development of embryo

ii) Energy for the biosynthetic process

iii) Nucleic acids for protein synthesis and embryonic development

Biochemical Changes

1. Nucleic acids

In monocots, during imbibition, there is a rapid decrease of DNA and RNA contents in the endosperm with a simultaneous increase in the embryonic axis. High concentration of RNA in the embryonic axis precedes cell division. Due to more cell division DNA content is increased.

2. Carbohydrates

Insoluble carbohydrates like starch are the important reserve food of cereals in the endosperm. During germination, starch is hydrolysed first into maltose in the presence of amylase and then maltose is converted into glucose by maltase. The glucose is further converted into soluble sucrose and transported to growing embryonic axis. During germination, the embryonic axis secretes gibberellic acid, into the aleurone layer which causes synthesis of amylase.

3. Lipids

Many plants like castor bean, peanut, etc, store large amount of lipids or fats as reserve food in their seeds. During germination, the fats are hydrolyzed into fatty acids and glycerol by lipase enzyme. Fatty acids are further converted into acetyl - COA by the process of oxidation. The acetyl COA is further converted into sucrose via glyoxylate cycle and is transported to the growing embryonic axis.

4. Proteins

Some plants store proteins as reserve food in their seeds. Proteins are hydrolysed into amino acids by peptidase enzyme. The amino acids may either provide energy by oxidation after deamination (removal of amino group) or may be utilized in the synthesis of new proteins.

5. Inorganic nutrients

A number of inorganic nutrients such as phosphate, calcium, magnesium and potassium are also stored in seeds in the form of phytin. These stored nutrients are liberated during germination due to the activity of various phosphatases including phytase.

Emergence of seedling out of the seed coat

All the changes described above gradually result in splitting of seed coat and emergence of the growing seedlings. First the radical comes out and grows downward, then plumule comes out and grows upward. Due to continued growth of this seedling, the plumule comes out of the soil, exposed to light and develops its own photosynthetic organs. Until the seedlings starts producing assimilate by its own photosynthetic organs, the reserve food available in the seed is sufficient to sustain the seedling growth (Fig. 11.1).

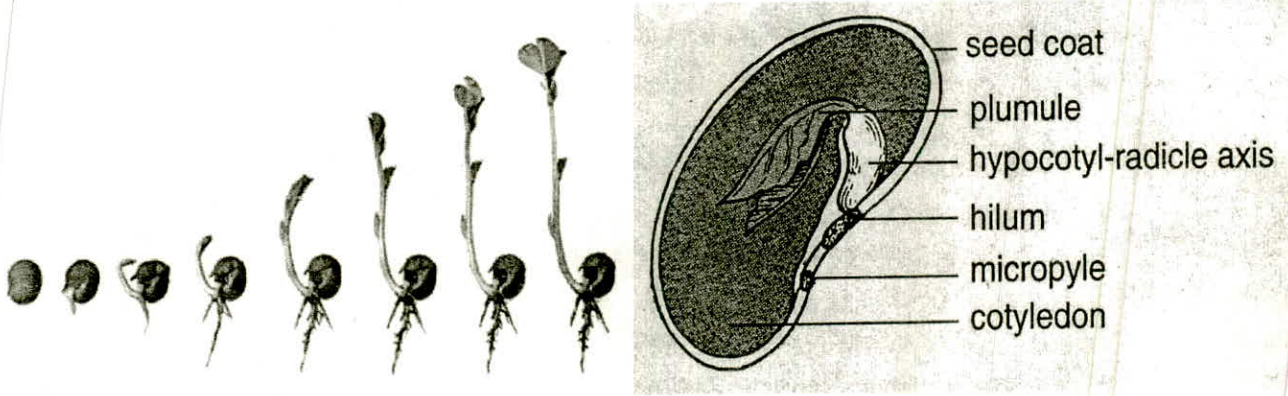


Figure 11.1 : Emergence of seedlings

Splitting of seed coat may take place either 1) by imbibitional pressure 2) by internal pressure created by the growing primary root 3) by hydrolytic enzymes which act ion cell wall contents of seed coat and digest it eg. Cellulase, pectinase etc and sometimes, the seed coat may be extensively damaged by the activity of micro-organisms in the soil.

Exercise 13: Seed treatments for breaking seed dormancy and Inducing vigorous seedling growth.

Materials Required: Seed, sand, stratification box (pit), sand paper, moss, hessian cloth, water, bavistin, labels, and glass containers

Procedure:

Methods of Breaking Dormancy:

What is Scarification?

Scarification is any process of breaking, scratching, mechanically altering or softening the seed covering to make them permeable to water and gases.Three types of treatments are commonly used as scarification treatments.

(a) Mechanical scarification:

- It is simple and effective if suitable equipment is available.
- Chipping hard seed coat by rubbing with sand paper, cutting with a file or cracking with a hammer are simple methods useful for small amount of relatively large seeds.
- For large scale, mechanical scarifiers are used. Seeds can be tumbled in drums lined with sand paper or in concrete mixers containing coarse sand or gravel.
- The sand gravel should be of different size than the seed to facilitate subsequent separation.

(b) Acid scarification:

- Dry seeds are placed in containers and covered with concentrated sulfuric acid (H_2SO_4) or HCl in the ratio of one part seed to two parts acid.
- The amount of seed treated at any time should be restricted to not more than 10kg to avoid uncontrollable heating.
- The containers should be of glass, earthenware or wood non metal or plastic. The mixture should be stirred cautiously at intervals during the treatment to produce uniform results.
- The time may vary from 10 minutes to 6 hours depending upon the species. With thick-coated seeds that require long periods, the process of scarification may be judged by drawing out samples at intervals and checking the thickness of the seed coat.
- When it becomes paper thin, the treatment should be terminated immediately.
- At the end of the treatment, the acid is poured off and the seeds are washed to remove the acid.
- The acid treated seeds can either be planted immediately when wet or dried and stored for later planting.
- Large seeds of most legume species respond to simple sulfuric acid treatment.

(c) Hot water scarification:

- Drop the seeds into 4-5 times their volume of hot water with temperature ranging from 77 to 100oC.
- The heat source is immediately removed, and the seeds soaked in the gradually cooking water for 12 to 24 hours.
- The unswollen seeds may be separated from the swollen seeds by suitable screens. The seed should be sown immediately after hot water treatment.

What is stratification?

Stratification is a method of handling dormant seed in which the imbibed seeds are subjected to a period of chilling to after ripen the embryo in alternate layers of sand or soil for a specific period. It is also known as moist chilling.

- The seeds can be planted immediately after stratification in the field.
- Seeds with a hard endocarp, such as Prunus spp. (the stone fruit including cherry, plum and peaches) show increased germination if planted early enough in the summer or fall to provide one to two months of warm temperature prior to the onset of chilling (Fig 13.1).

Refrigerated stratification:

Procedure

- An alternative to outdoor field stratification is refrigerated stratification. It is useful for small seed lots or valuable seeds that require special handling.
- Dry seeds should be fully imbibed with water prior to refrigerated stratification. Twelve to twenty four hours of soaking at warm temperature may be sufficient for seeds without hard seed coats.
- After soaking seeds are usually mixed with well washed sand, peat moss or vermiculite. A good medium is a mixture of one part coarse sand to one part peat, moistened and allowed to stand 24 hours before use.
- Seeds are placed in alternate layers of sand or medium. The usual stratification temperature is 0-10oC.
- At higher temperature seeds sprout prematurely and low temperature delay sprouting.
- During stratification seeds should be examined periodically, if they are dry, the medium should be remoistened.
- The stratified seed is separated from the medium prior to sowing in nursery beds.

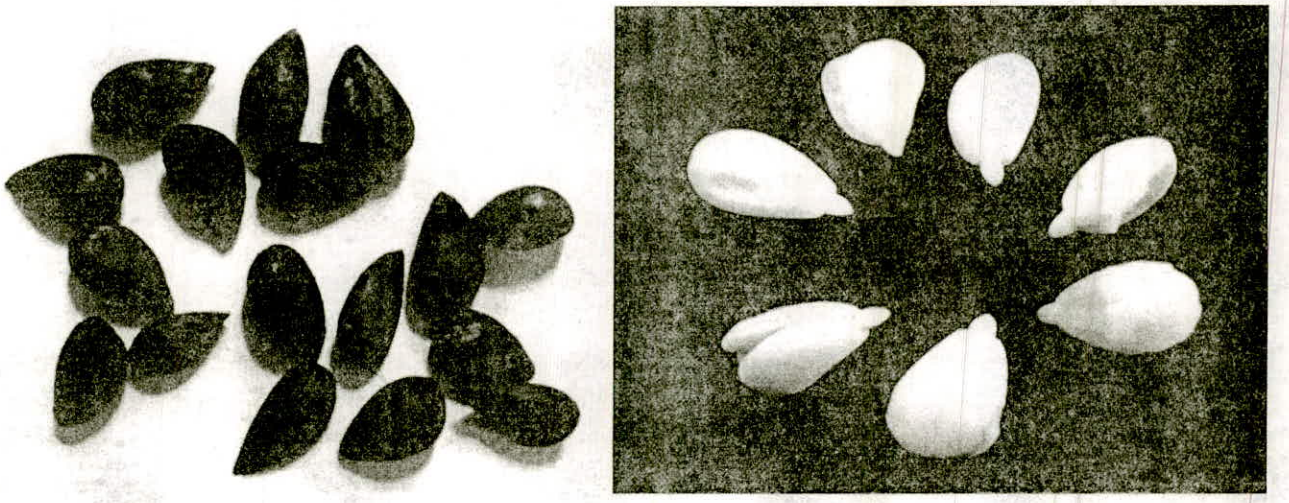


Figure 13.1 : (a) Stratified seed of apple **(b) Stratified seed of apple(inside view)**

- The stratification of seeds results in quick and uniform germination and therefore the seed should be subjected to stratification invariably under all conditions.

Stratification period and percentage of germination in seeds of temperate fruit crops:

Kinds of Fruits	Stratification period (days)	Germination (%)
Apple	70-75	70-75
Kainth (Pyrus patia)	30-35	90-95
Peach	60-70	55-60
Apricort	45-50	75-80
Almond	45-50	85-90
Walnut	95-100	80-85
Pecan	70-75	75-80

Precautions:

- Scarification should not proceed to the point at which the seeds are injured and inner parts of seed are exposed.
- The sand should be passed through coarse sieve mesh to separate bigger size gravels so as to avoid confusion between seed and gravels at the time of sowing.
- Irrigation during stratification should be given at regular intervals to maintain adequate moisture level.
- Seed should not sprout during stratification.
- Mulching of seed beds after sowing is must to ensure uniform germination

Exercise 14: Physiology of ripening of fruits.

What is fruit ripening?

After a period of growth, fruit undergoes some characteristic qualitative changes leading to edible state. These changes are collectively referred to as fruit ripening. Some important events in fruit ripening are as follows:

1. Changes with ripening.
2. The respiratory climacteric
3. Hormonal controls of ripening.

1. Changes with ripening

The general changes that occur during the process of ripening of fruits are

(a.) Softening of fruit (b.) Hydrolytic conversion of complex storage materials into simpler forms

(c.) Changes in pigments and flavours.

Softening is an important change with the ripening of fruits. The major role played in this process is that of cell wall degrading enzymes, associated with hydrolysis of cell contents. As such pectolytic enzyme activities induces solubilization of pectic substances found in middle lamellae.

- Hydrolytic changes in the fruit during ripening usually lead to the formation ofsugars. Such changes show different rates in different fruits, e.g. banana ripens extremely fast, apple shows gradual ripening and citrus fruits show very slow changes.
- During ripening of fruits, some qualitative changes occur such as change in pigmentation, production of flavour and depletion of astringent substances.
- The changes in pigments in fruits are normally the loss of chlorophyll and the development of carotenoids. There may be changes in colour due to moderate loss of chlorophyll with little or no formation of carotenoids as in banana or due to complete formation of carotenoids, as in oranges.
- The newly developed pigments may be carotenes as in papaya or anthocyanins as is strawberry and these are synthesized in the presence of sunlight and with the involvement of phytochrome.

2. The respiratory climacteric

Kidd and West (1930) observed that in apple fruits, a major change occurs in respiration rates during their ripening. They found lowering of respiration rate in maturing fruits followed by large increase in respiration during ripening. And after reaching a climacteric peak, the rate of respiration falls.

As regards occurrence of climacteric, fruits may be divided into two types

Climacteric fruits : In climacteric fruits, the rate of respiration is higher during their ripening.

Examples: Apple, Apricot, Banana, Custard apple, Mango ,Strawberry Water Melon,Olive Peach, Plum, Tomato.

Non climacteric fruits: In non-climacteric fruits, the rate of respiration remains steady during their ripening. Examples: Pear, Persimmon, Pineapple, Grapes, Chillies, Orange, Avocado, Lemon, Mandarins, Sapota, Watermelon, Fig.

3. Hormonal control of ripening

Ethylene has been established as a ripening hormone. Massive doses of ethylene can bring about ripening changes in immature fruits. So, it is the hormone which plays the most powerful regulatory role in ripening. It has been observed that a rise of ethylene level occurs at the onset of the climacteric rise and can be assigned the role of the trigger of ripening.

Symptoms of Fruit Ripening

1. Texture (Softening of fruit)

The changes in the texture of fruit result due to changes in the structure and composition of their cell walls.

2. Colour

The factors responsible for changes in colour of fruit during ripening may be due to changes in pigments localized in chloroplasts or those which are stored outside chloroplasts in vacuoles.

(a) Colour changes due to conversion of chloroplasts into chromoplasts - The Carotenoids:

A major factor in the colour changes of fruit ripening is the transition fromchloroplasts which are rich in green pigment chlorophyll into chromoplasts which are rich in red or yellow carotenoid pigments.

(b) Colour changes due to pigments stored outside chloroplasts (i.e., in vacuole)-The anthocyanins.

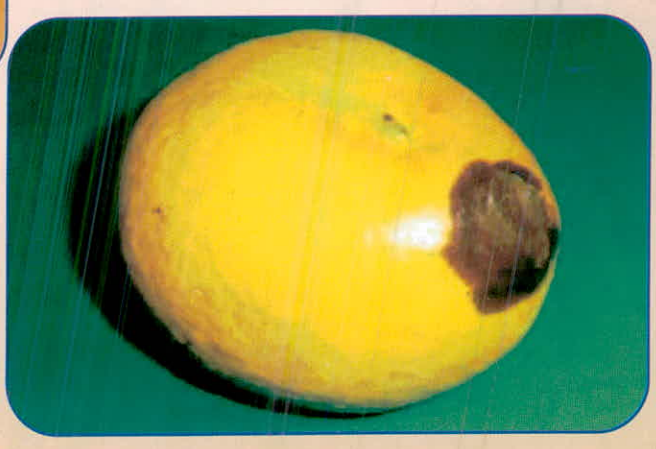
Anthocyanins are water soluble phenolic pigments which accumulate in vacuole and impart red, blue and purple colors to many fruits such as ripening fruits of apple, grape, strawberry etc. Anthocyanins exist as complex conjugates of parent aglycones called as anthocyanidins.

PHYSIOLOGICAL DISORDERS IN DIFFERENT FRUIT CROPS:

Vegetative malformation of mango

Floral malformation of mango

Spongy tissue of mango



Black tip of mango

PHYSIOLOGICAL DISORDERS IN DIFFERENT FRUIT CROPS:

Finger drop in banana

Choke rot in banana

Boran deficiency in banana

Ca deficiency in banana

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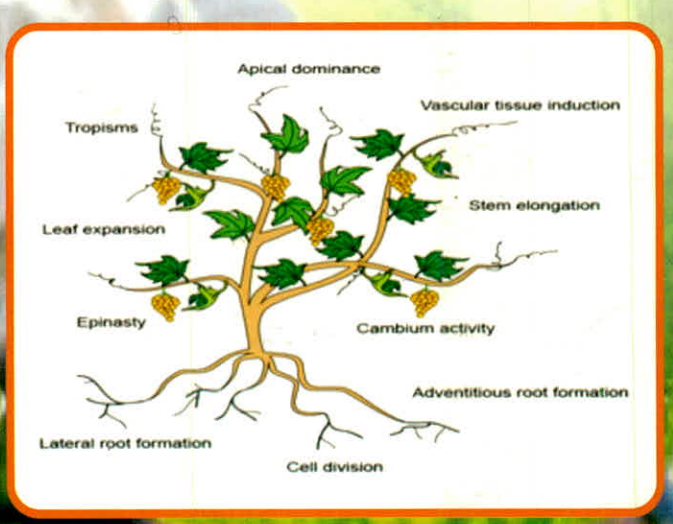

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Edition: 2013 (First)

Published by
Dean
College of Horticulture,
Indira Gandhi Krishi Vishwavidyalaya
Rajnandgaon - 431 445

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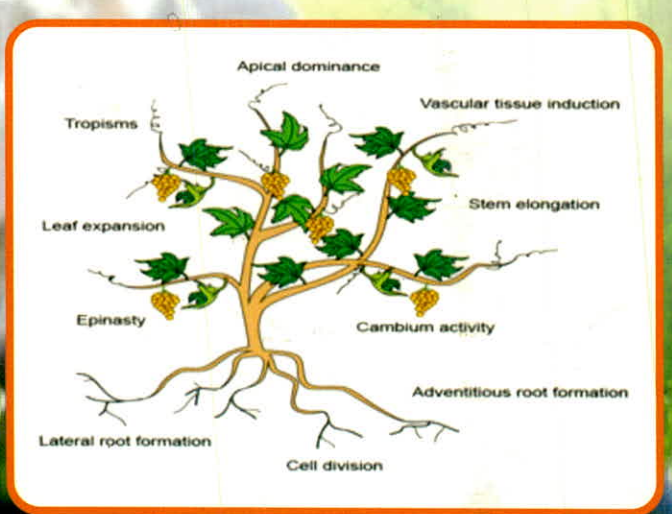

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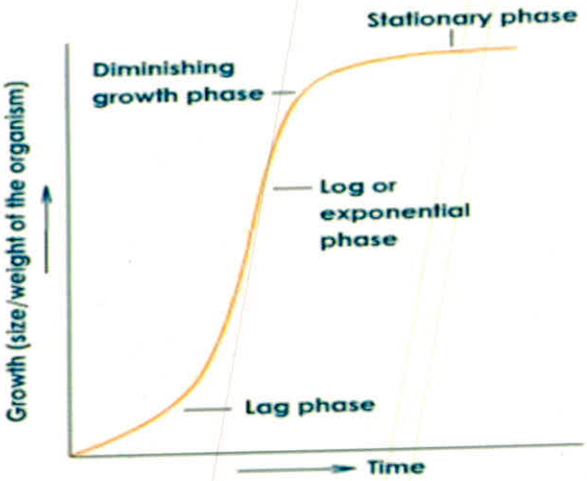
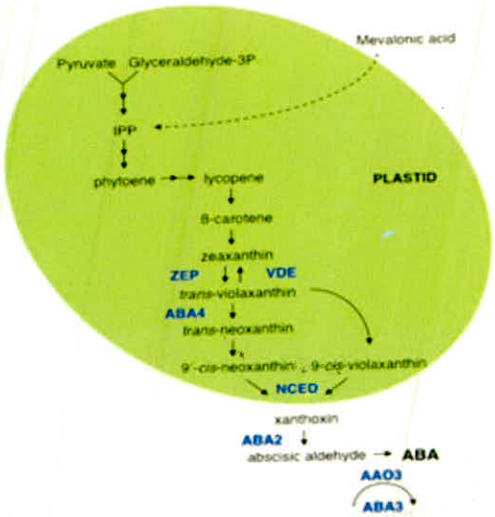
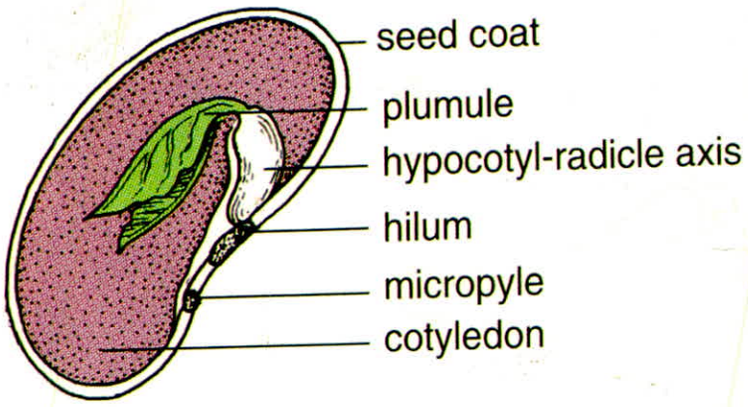






PHYSIOLOGICAL DISORDERS IN DIFFERENT FRUIT CROPS

Chilling injury in banana

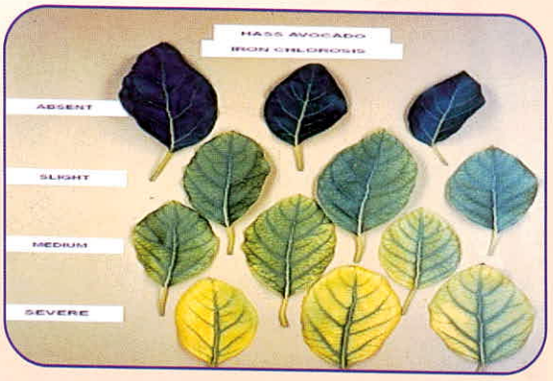
K deficiency in banana

Maturity Bronzing

N deficiency in banana

Zn deficiency in citrus

Zn deficiency in banana



Fe chlorosis in Acid lime

Hen and chicken disorder of grape