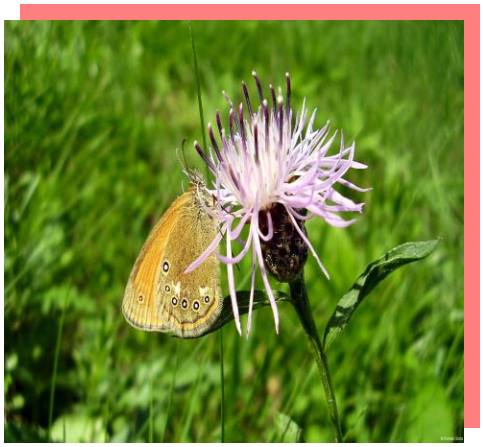
Growth and Development of Horticultural Crops



8. Growth and Development of Horticultural Crops (HPH 101) 2(1+1)

Growth and development-definitions, components, photosynthetic productivity, leaf area index (LAI) - optimum LAI in horticultural crops, canopy development; different stages of growth, growth curves, growth analysis in horticultural crops. Plant bioregulators- auxin, gibberellin, cytokinin, ethylene inhibitors and retardants, basic functions, biosynthesis, role in crop growth and development, propagation, flowering, fruit setting, fruit thinning, fruit development, fruit drop, and fruit ripening. Flowering-factors affecting flowering, physiology of flowering, photoperiodism-long day, short day and day neutral plants, vernalisation and its application in horticulture, pruning and training physiological basis of training and pruning source and sink relationship, translocation of assimilates. Physiology of seed development and maturation, seed dormancy and bud dormancy, causes and breaking methods in horticultural crops. Physiology of fruit growth and development, fruit setting, factors affecting fruit set and development, physiology of ripening of fruits-climatic and nonclimacteric fruits.

***Practical**: Estimation of photosynthetic potential of horticultural crops, leaf area index, growth analysis parameters including harvest index, bioassay of plant hormones, identification of synthetic plant hormones and growth retardants, preparations of hormonal solution and induction of rooting in cuttings, ripening of fruits and control of flower and fruit drop. Important physiological disorders and their remedial measures in fruits and vegetables, rapid tissue test, seed dormancy, seed viability by tetrazolium test, seed germination and breaking seed dormancy with chemicals and growth regulators.*

Lecture No.1

Growth

Growth is defined as an irreversible increase in size and it may be evaluated by measurements of mass, length or height, surface area or volume. Growth is restricted only to living cells and is accomplished by metabolic processes involving synthesize of macromolecules, such as nucleic acids, proteins, lipids and polysaccharides at the expense of metabolic energy.

Growth at cellular level is also accompanied by the organization of macromolecules into assemblages of membranes, plastids, mitochondria, ribosomes and other cell organelles. Cells do not definitely increase in size but divide, giving rise to daughter cells. An important process during cell division is synthesis and replication of nuclear DNA in the chromosomes, which is then passed into the daughter cells. Therefore, the term growth is used to denote an increase in size by cell division and cell enlargement, together with the synthesis of new cellulose materials and the organization of cellulose organelles.

Growth is also defined as a vital process which brings about a permanent change in any plant or its part in respect to its size, form, weight and volume.

Patterns of Growth and Development

Some Features of Plant Growth

Growth in plants is restricted to certain zones, recently produced by cell division in a **meristem**. It is easy to confuse growth (as defined above as an increase in size) with cell division in meristems. Cell division alone does not cause increased size, but the cellular products of division also increase in volume and cause growth. Root and shoot tips **(apices)** are meristematic in nature.

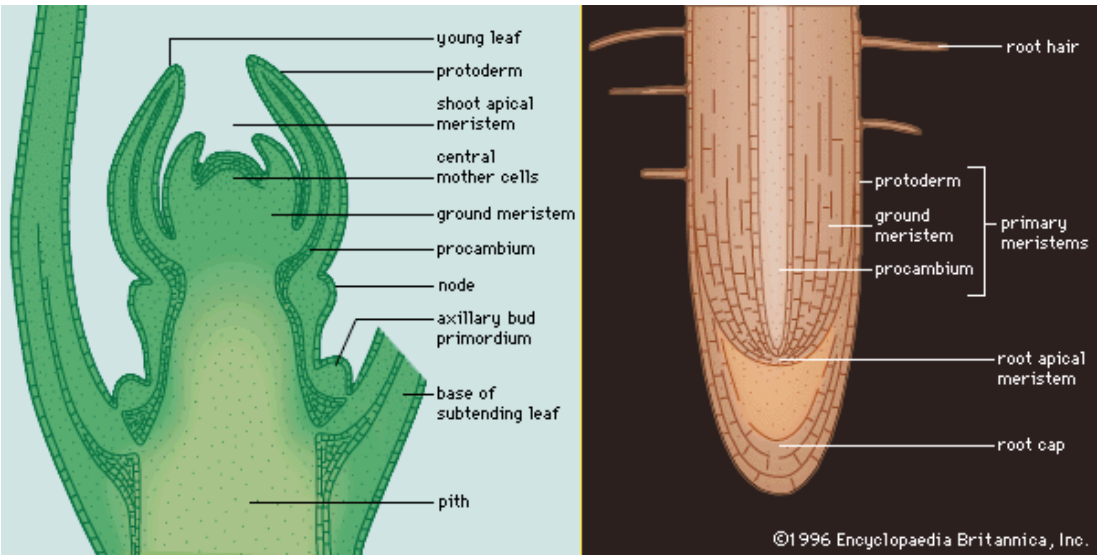


Fig 1. Shoot system with apical meristem Root system with apical meristem

http://media-2.web.britannica.com/

Other meristematic tissues are found in the vascular cambium and just above the nodes of monocots or at the bases of grass leaves. The root and shoot apical meristems are formed during embryo development, while the seed develops and are called **primary meristems**. The vascular cambium and the meristmatic zones of monocot nodes and grass leaves are indistinguishable until after germination; they are **secondary meristems**.

Some plant structures are determinate; it grows to certain size and then stops, eventually undergoing senescence and death. Leaves, flowers, and fruits are good examples of determinate structures. On the other hand, the vegetative stem and root are indeterminate structures. They grow by meristems that continuously replenish themselves, remaining live. A bristlecone pine that has been growing for 4,000 years could probably yield a cutting that would form roots at its base, producing another tree that might live for another 4,000 years. At the end of the time, another cutting might be taken and so on, potentially forever; that is, plants can be cloned from individual parts. Some fruit trees have been propagated from stem sections for centuries.

Although indeterminate meristems can be killed, it is potentially immortal. But death is the ultimate fate of determinate structures. When an indeterminate, vegetative meristems becomes reproductive (that is, begins to form a flower), it becomes determinate.

Although there are borderline cases, entire plants are in a sense either determinate or indeterminate. We use different terms, however; monocarpic species flower only once and then die; polycarpic species after flowering, return to a vegetative mode of growth, and flower at least once more before drying. Most monocarpic species are annuals (live only one year), but there are variations in them. Many annuals germinate from seeds in the spring, grow during the summer and autumn, and die before winter, perpetuating themselves only as seeds. Spring wheat and rye are commercial annuals that are planted in the spring, but seeds of winter wheat or rye germinate in the fall, over winter as seedlings beneath the snow, and flower the next spring.

Typical **biennials**, such as beet (*Beta vulgaris*), carrot (*Daucus carota*), and henbane (*Hyoscyamus niger*) germinate in the spring and spend the first season as a vegetative rosette of leaves that dies back in late fall. Such a plant overwinters as a root with its shoot reduced to a compressed apical mersitem surrounded by some remaining protective dead leaves (meristem plus leaves is called a **perenniating bud)**. During the second summer, the apical meristems form stem cells that elongate bolt into a flowering stalk.

The century plant (*Agaves americana*) may exist for a decade or more before flowering and once attain flowering, it may die. Though a monocarpic species, it would be called a perennial because it lives for more than two growing seasons. Bamboos (Bambusa and other genera), which may live more than half a century before flowering, die after flowering, is an excellent example of the extreme monocarpic growth habit. Polycarpic plants, perennials by definition, do not convert all their vegetative meristems to determinate reproductive ones. Woody perennials (shrubs and trees) may use only some of their axillary buds for the formation of flowers, keeping the terminal buds vegetative; alternatively, terminal buds may flower while axillary buds remain vegetative. Sometimes, a single meristem forms only one flower, as in a tulip, whereas single grass or Asteraceae meristem forms an inflorescence or head of flowers (for example, sunflower, bottle brush, *Callistemon* sp.)

Kinetics of growth – the course of growth (Grand period of growth) or sigmoid curve (Grand period curve)

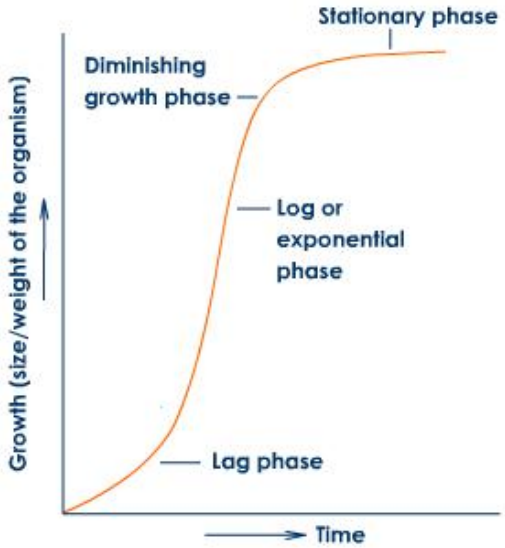


Fig 2. Sigmoid growth curve

http://www.tutorvista.com

Usually 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 period of Growth**. If this growth rate is plotted against time, a slanting S shaped curve is obtained which is called as **Sigmoid Curve or Grand Period Curve**.

The sigmoid curve represents the integrated sum of the curves for each growing organ and cell and presents the changing size of these parts. Similarly, when dry weight is measured as an index of growth before maturity, the curve takes the well known sigmoid form. Environmental conditions may alter growth rates but not the sigmoid form of the growth curve.

In unicellular organisms such as *Chlamydomonas* or bacteria, growth is assessed by a count of number of cells per milliliter at increasing times after the cells are placed in a fresh nutrient medium and under environmental conditions (light, temperature, etc.) suitable for

optimal growth. Here also, there is initial lag period during which cells activate their biochemical machinery for rapid growth by synthesizing necessary enzymes. This is followed by a time period during which there is exponential increase in cell number which is called as **log period**. This period of rapid growth does not continue indefinitely and due to depleted nutrient supply, accumulation of toxic products and other limiting factors ultimately leads to **decreasing cell number** until the population of cells reaches a **steady state** in which the number of cells remains **constant (stationary) or even declines**. If number of cells per milliliter is plotted against time (hours), again a sigmoid curve is obtained as described earlier.

Lecture No. 2
Growth factors and growth correlation

Growth factors

Internal Factors and Environmental Factors

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 microorganisms.

Limitation of growth factors

a) Liebig law of minimum

This concept was formulated by German chemist Justus von Liebig, often called the "father of the fertilizer industry". This is an example of Liebig's Law of the Minimum, which states that plant growth will continue as long as all required factors are present (e.g. light, water, nitrogen, phosphorus, potassium etc.). When one of those factors is depleted, growth stops. Increasing the amount of the "limiting" component will allow growth to continue until that component (or another) is depleted.

The nutrient most typically "limiting" algae growth in lakes is phosphorus. If phosphorus concentrations can be maintained, then algal growth would be controlled. In Mark Twain Lake, (the largest reservoir in northern Missouri), light is the factor that most often limits algae. (http://www.lmvp.org)

A deficiency or absence of any one necessary component, when all others are present, renders the soil barren for crops for which that nutrient or factor is needed. It is also called as "barren concept".

b) Blackman's law of limiting factors (1905)

The law states that “When a process is conditioned as to its rapidity by a number of separate factors, the rate of process is limited by the pace of the slowest factor”. To explain the principle of limiting factor, Blackman gave the following illustration which is also shown graphically in Fig. 3

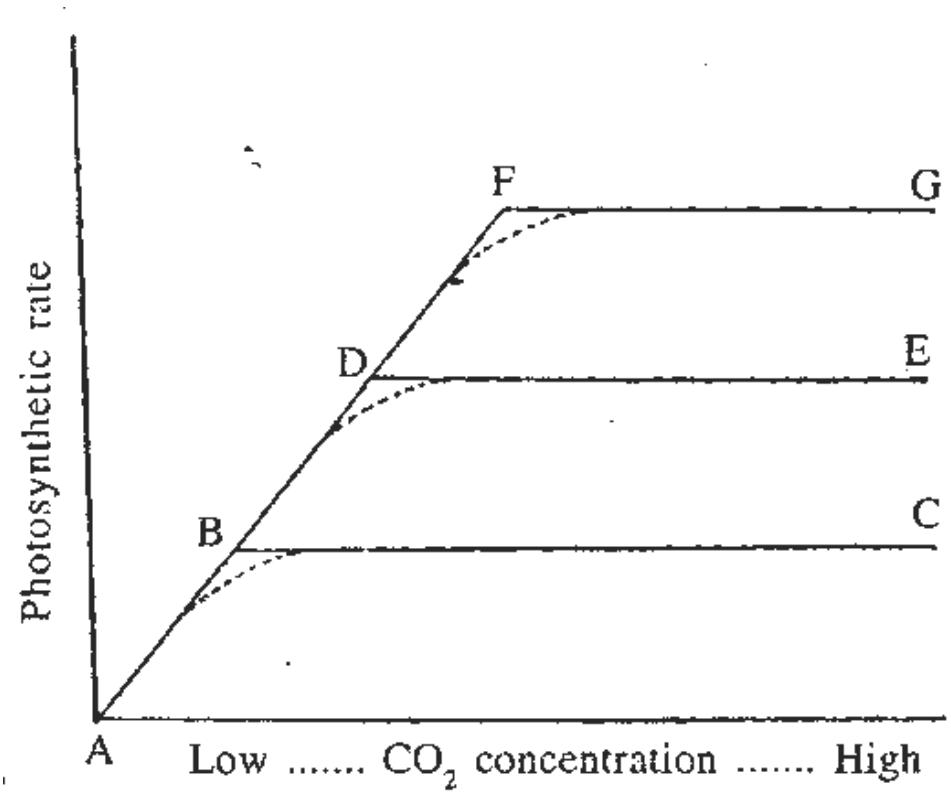


Fig 3. Graphic representation of Blackman’s law of limiting factors

Suppose a leaf is exposed to a certain light intensity which allows the leaf to utilize 5 mg of CO_2 per hour in photosynthesis. The photosynthesis will not occur if the CO_2 is totally absent

in the atmosphere. If one mg of CO_2 is available, the rate of photosynthesis is limited due to CO_2 factor. If the CO_2 concentration is increased in the atmosphere from 1 mg to 5 mg / hour, the rate of photosynthesis increases along the line AB. Thus, the increase in photosynthetic rate will be proportionate with the increase in CO_2 concentration up to 5 mg. Any further increase in amount of CO_2, will not increase the rate of photosynthesis and the rate becomes constant along the line BC. This is because the light factor (low intensity) has now become the limiting factor. Addition of CO_2 with increase in light intensity increases the rate of photosynthesis along the line BD. Under these conditions increase of CO_2 will not increase the rate of photosynthesis. The rate becomes constant along the line DE. Here also the light intensity becomes the limiting factor. Further, increase in light intensity from the medium to high, increases the rate of photosynthesis along the line DF by adding CO_2. When the rate attains maximum at F, further, increase in CO_2 will not increase the rate of photosynthesis, which becomes constant along the line FG. It indicates that sunlight again becomes the limiting factor. Besides, light and CO_2, other factors such as temperature, water, etc may also become limiting under certain conditions.

c) Mitscherlich: law of diminishing returns

Mitscherlich (1909), a Soil Scientist observed that when plants had adequate amounts of all but one limiting element, the growth response is proportional to the limiting element, however plant growth increased with additional increments, of a limiting factor, but not in direct proportion. The law of diminishing return states that the "increase in any crop produced by a unit increment of a deficient factor is proportional to the element of that factor from the minimum". The response is curvilinear, instead of linear, as Blackman suggested.

Growth correlations

Plants acquire a characteristic shape or form by correlated growth of component parts. Favorable environment can enhance growth quantitatively, but the geometry of the parts and the whole plant is relatively constant. The relationship between the growth rate of individual parts of an organ or organism is called as **allometry.**

The relationship between two variants (X and Y) may be expressed as

$$Y = bx^k$$

Where

X and Y are physical parameters

b and k are constant. K, being the allometric constant.

The quantity of K can be calculated from the equation log Y = log b + k log 2 or by linear regression analysis of the data set Y and X. For eg. If length and breadth of a leaf expand at the same rate, the slope of the regression line (the coefficient of allometry or K) is 1.0 (one). Similarly the harvest index (proportion of seed weight to whole plant weight) has a relatively high coefficient of allometry. Actually allometry deals with the correlation of physiological process, specifically with reference to growth analysis of crops.

Shoot-root ratio:

It is a type of allometric growth that reflects one type of tolerance to drought stress. Though the shoot root ratio is under genetic control, it can be modified by the environment. For example, under high nitrogen regime approximately 90per cent of the photosynthate is partitioned into shoot compared to 50 per cent to the shoot under low N. Similarly the water deficits significantly affect the shoot growth.

Apical and lateral growth: Plants assume a characteristic form or geometry mainly due to apical and lateral buds growth.

Vegetative and reproductive growth

In annuals, vegetative growth is generally terminated by reproduction. Leaves, stems and other vegetative parts not only fail to compete for current assimilate during ripening of fruits but also sacrifice previously accumulated carbon and minerals through mobilization and redistribution.

Perennials make only partial commitment to reproduction, and shoot that bear fruits may remain healthy and new vegetative shoots are generated from axillary buds.

Growth and differentiation

Plant development is a combination of complex processes of growth and differentiation that leads to an accumulation of dry matter.

While growth is the increment in weight or volume due to cell division and cell enlargement, differentiation is the formation of specialized cells like xylem and phloem and formation of different plant parts like root, stem, leaves, etc.

Differentiation processes have three requisites

i. Available assimilates in excess of most metabolic uses

ii. A favourable temperature

iii. Proper enzyme system for cell wall thickening, secondary product accumulation (eg. alkaloids and starches) and protoplasm hardening may occur which result in changes in anatomy and morphology.

Lecture No.3

Growth analysis

The analysis of yield influencing factors and plant development as net photosynthate accumulation integrated over time is known as **growth analysis**. Growth analysis can be made at individual plant level or plant communities. The analyses made at individual plant level are RGR, AGR, NAR, LAR, SLA, SLW and allometry (shoot/root ratio). The analyses made at plant community level are LAI, LAD, CGR and DMA.

The technique of growth analysis is advantageous to crop scientist as it helps to find out the relationship between photosynthetic production and rate of increase in dry matter. Methods of growth studies provide better understanding of growth processes and limitations of the crop yield.

Parameters arrived through 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 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 cm^2g^{-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

$$LAD = \frac{L_1 + L_2}{2} \times (t_2 - t_1)$$

LAI (i) – Leaf area index at first stage

LAI (ii) - Leaf area index at second stage

$t_2 - t_1$ – 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, Radfort (1967) suggested leaf area ratio as a measure of leaf area to the weight of the whole plant. It is expressed as $cm^2\ g^{-1}$.

$$LAD = \frac{L_1 + L_2}{2} \times (t_2 - t_1)$$

In broad sense, LAR represents the ratio of photosynthesizing to respiratory material within the plant.

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}

$$NAR = \frac{\log_e L_2 - \log_e L_1}{L_2 - L_1} \times \frac{W_2 - W_1}{t_2 - t_1}$$

Where

Log e L_2 = Natural log of leaf area at stage 2.

Log e L_1 = Natural log of leaf area at stage 1.

L_2 & L_1 = Leaf area at stage 2 & 1 respectively

W_2 & W_1 = Dry weight of the whole plant at stage 2 & 1 respectively

t2-t1 = Time interval between the two stages

NAR is expressed as mg cm^{-2} day^{-1}

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.

$$RGR = \frac{\log_e W_2 - \log_e W_1}{t_2 - t_1}$$

W_2 & W_1 = Whole plant dry weight at t_2 & t_1 respectively and expressed as g g^{-1} day^{-1}

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 following formula to arrive Crop Growth Rate

$$CGR = \frac{(W_2 - W_1)}{(t_2 - t_1)} \times \frac{1}{P}$$

Where, W_2 and W_1 are total plant dry weight at time t_2 and t_1 and P is plant population per unit area.

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

CGR = LAI X NAR

CGR increases as LAI increases to an optimum because of greater light interception.

CGR is expressed as mg m^{-2} day^{-1}.

12) Harvest Index (HI)

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

Lecture No. 4

Plant Growth Hormones

Most of the physiological activities and growth in plants are regulated by the **action and interaction** of some chemical substances in them called as **hormones** and by certain naturally occurring **inhibitors** *e.g.,* phenols, flavonols and abscisic acid. To distinguish the plant hormones from the animal hormones they are termed as **phytohormones**. According to Pincus and Thimann (1948), a plant hormone is defined as **"organic substance produced naturally in the higher plants, controlling growth or other physiological functions at a site remote from its place of production and active in minute amounts."**

These phytohormones have also been termed as growth hormones, growth promoting substances, growth substances, growth factors, growth regulators etc., by various workers and defined accordingly. 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 and absicisic acid (ABA)** and more recently **brassinosteroids** have also acquired status of natural plant growth hormones.

Auxins

Discovery and Chemical Nature

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 agar-blocks 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.**

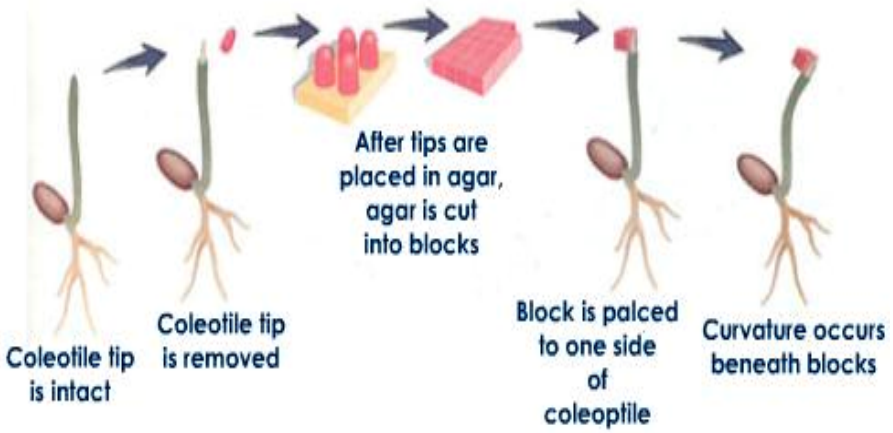


Fig.2 ***Avena*** **Curvature Test.**

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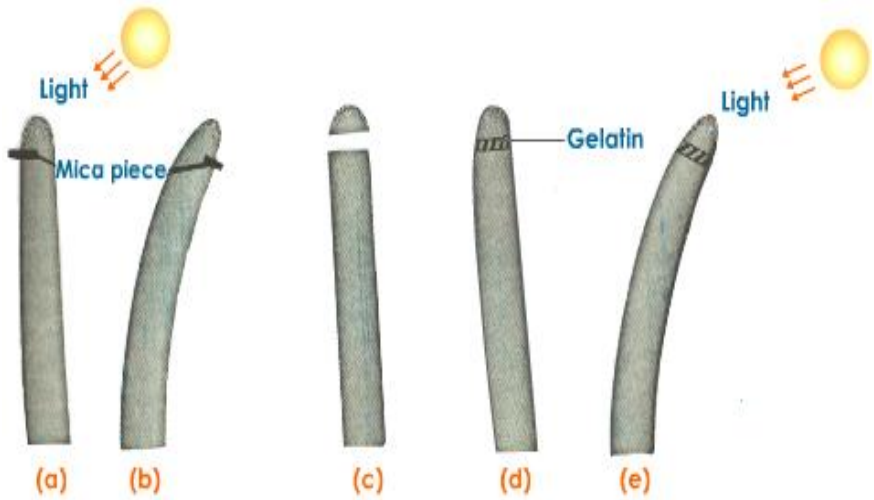

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Avena test (a) A piece of mica inserted on the shaded side prevented curvature of the coleoptile, (b) but not when it was inserted on the illuminated side, (c) when the tip

was removed (d) but was put back with a block of gelatine, (e) normal phototropic curvature occurred.

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 several other bioassay techniques. 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 commonly used 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 is used as a rooting hormone for certain plants. Another biologically active synthetic auxin lacking 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 effects 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.

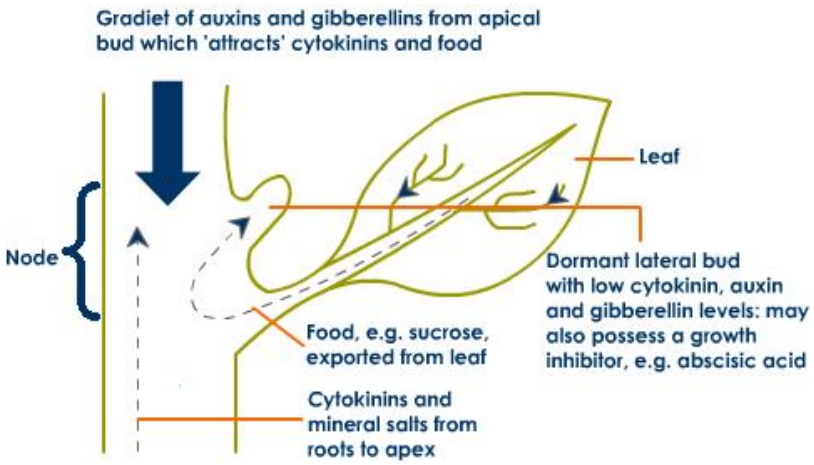

Fig.3 Possible Involvement of Plant Growth Substances in Apical Dominance in Presence of Apical Bud

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3. Root Initiation

In contrast to the stem, the higher concentration of auxin inhibits the elongation of root but the number of lateral branch roots is considerably increased i.e., the higher conc. of auxin initiates more lateral branch roots.

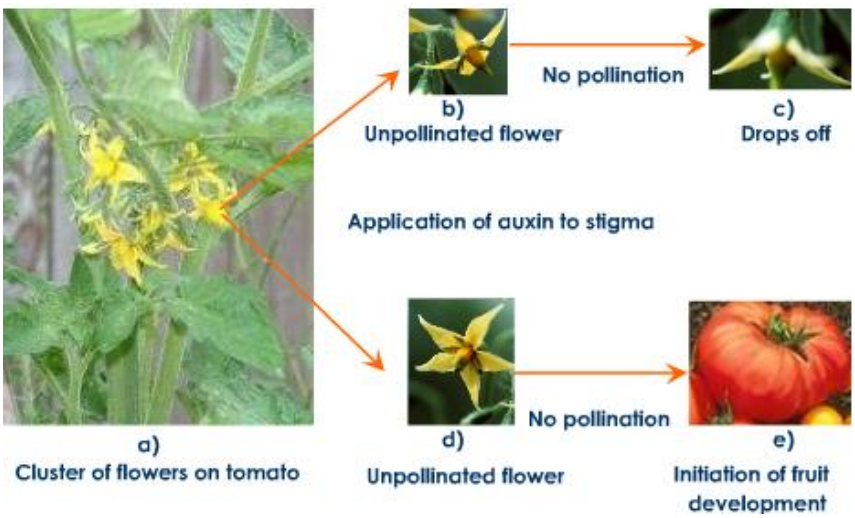
Application of **IAA** in lanolin paste to the cut end of a young stem resulted in an early and extensive rooting. This fact is of great practical importance and has been widely utilized to promote root formation in economically useful plants which are propagated by cuttings.

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.



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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.

Distribution of auxin (IAA) in plant

Auxin (IAA) is widely distributed in plant but relative concentrations differ in different parts of the plant. Since auxin is synthesized in growing tips or meristematic regions of the plant from where it is transported to other plant parts, the highest concentrations of the auxin are found in these parts such as growing shoot and root tips, young leaves and developing axillary shoots.

Distribution of auxin in monocot and dicot seedlings:

In monocot seedling, the highest concentration of auxin is found in the coleoptiles tip which decreases progressively toward its base. From the base of the coleoptiles, the auxin concentration increases progressively up to the root tip. However, the concentration of auxin at the tip of root is much lower than at the coleoptiles tip.

In dicot seedling, although the pattern of auxin distribution appears to be complex, but obviously highest auxin concentrations are found in growing regions of shoot, root, young leaves and developing axillary shoots.

Within the plant, the auxins may be present in two forms-free auxins and bound auxins. Free auxins are those which can be easily extracted by various organic solvents such as diethyl ether or those which are easily diffusible such as that obtained in agar block from cut coleoptiles tip. Bound auxins on the other hand, need more drastic methods for their extraction from plants such as hydrolysis, autolysis, enzymolysis etc., and are not easily diffusible. Bound auxins occur in plant as complexes (conjugated auxins) usually with carbohydrates such as glucose, arabinose or sugar alcohols, or proteins or amino acids such as aspartate, glutamate or with inositol.

- The **free form** of auxin is **biologically active** form of the hormone. In **bound** or **conjugated form** (which predominates in plants), the auxin is considered to be biologically inactive.
- The metabolism of bound or conjugated auxin might be a major contributing factor in controlling level of free auxin in plants.

Biosynthesis of Auxin (IAA) in plants

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.

(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**.

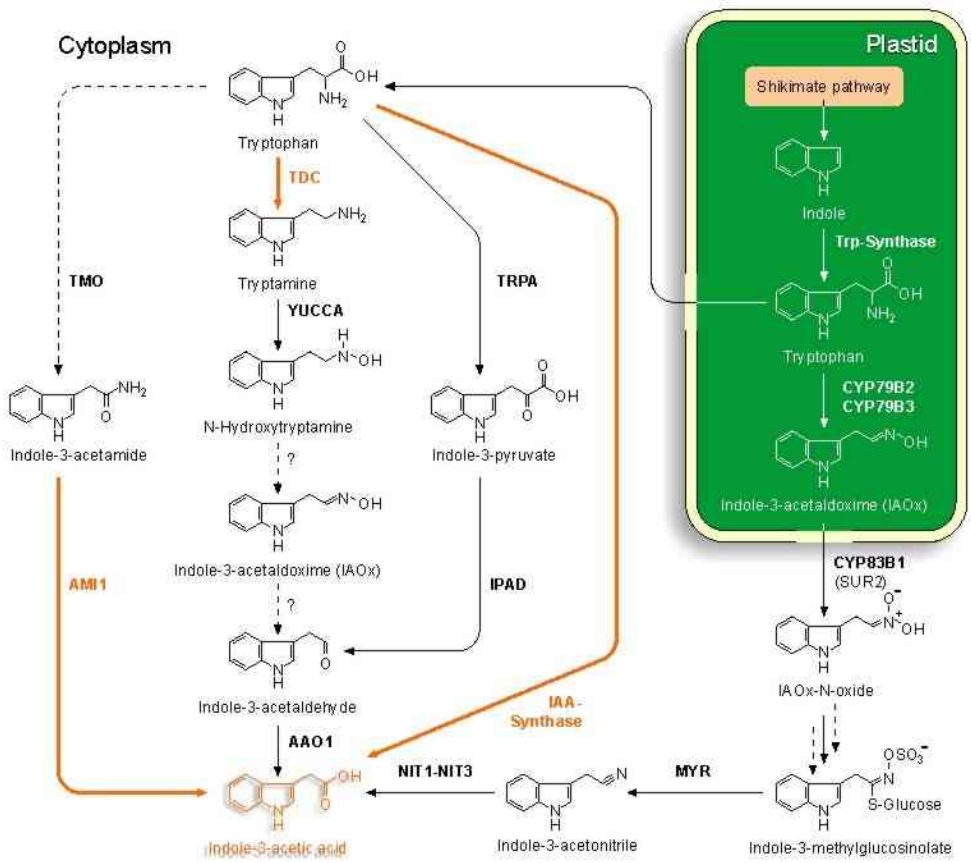
(b) IPA (Indole-3-pyruvic acid) pathway

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.

- One of the above two methods (sometimes both) is most common pathway of formation of IAA in plants.

(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 the presence of the enzyme nitrilase. Indole-3-acetaldoxime and indole-3-acetaonitrile (IAN) are the intermediates



Pathways of tryptophan-dependent indole-3-acetic acid biosynthesis.

http://www.ruhr-uni-bochum.de/sfb480/Bilder%20Teilprojekte/A%2010/Figure%201.jpg

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.

Destruction / inactivation of auxin in plant

Sufficient levels of auxin in plant required for regulation of plant growth are maintained not only by the synthesis of auxin, but also by its destruction or inactivation.

Chief method for the destruction (degradation) of auxin in plant is its oxidation by O_2 in the presence of the enzyme **IAA-oxidase** or **peroxidase**. This oxidation involves removal of CO_2 from the carboxylic group of auxin (IAA) and results in the formation of a variety of compounds, but **3-methyl-oxindole** is the major end product.

Auxin may be temporarily inactivated in plants by its conversion into its bound form (bound auxin or conjugated auxin) in which auxin is conjugated to a variety of substances such as carbohydrates, amino acides, proteins or inositol etc.

Rapid inactivation of auxin may occur by irradiation with X-rays and gamma rays, Ultra violet light is also known to reduce auxin levels in plants, Inactivation or decomposition of IAA by light has been called as **Photo-oxidation/ oxidation by O_2.**

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 effects 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.

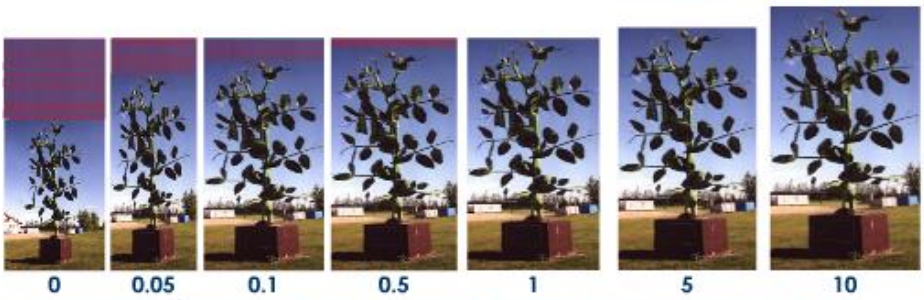
   In potatoes also, there is a dormant period after harvest, but the application of gibberellin sprouts the eyes vigorously.

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.



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.

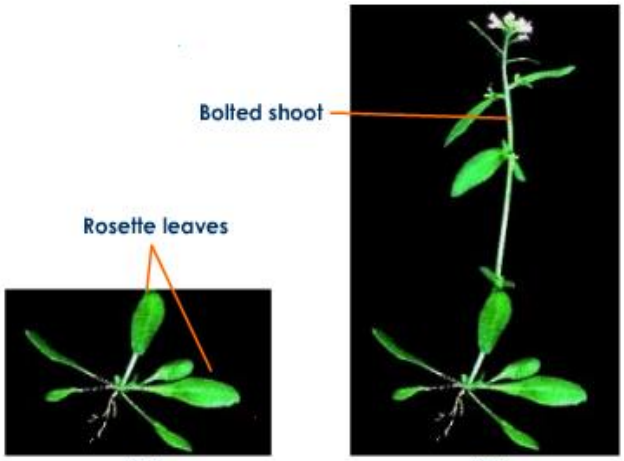
http://image.wistatutor.com/content/plant-growth-movements/gibberellins-dwarf-pea-assay-principle.jpeg

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* (It is a Long Day Plant*) by the application of gibberellins even under non-inductive short days.

In *Hyoscyamus niger* (also a Long Day Plant) gibberellins treatment causes bolting and flowering under non-inductive short days. While in Long Day Plants the gibberellins treatment usually results in early flowering, its effects are quite variable in Short Day Plants. It may either have no effect, or inhibit, or may activate flowering.



Showing bolting in a 'rosette' plant (cabbage) induced by the application of gibberellin.
a() an untreated plant (b)Treated plant showing bolting.
Note the elongated internodes and flowering.

http://image.wistatutor.com/content/plant-growth-movements/bolting-rosette.jpeg

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

It is common observation that the dark grown plants become etiolated and have taller, thinner and pale stems while the light grown plants have shorter, thicker and green stems, and it may be concluded that light has inhibitory effect on stem elongation. 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-α-Amylase

One of the important functions of gibberellins is to cause *de novo* synthesis of the enzyme α-**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.

Distribution of gibberellins in plants

Gibberellins are found in all parts of higher including shoots, roots, leaves, flower, petals, anthers and seeds. Gibberellins activity has also been shown in plastids. In general, reproductive parts contain much higher concentrations of gibberellins than the vegetative parts. In growing embryos after fertilization, cell division takes place vigorously, aided by auxin followed by cell expansion. Gibberellins are responsible for cell wall loosening and cell enlargement. These enlarged cells import assimilates from the source for storage in the reproductive organs. Hence, gibberellins play major role in increasing yields of seeds and fruits. Immature seeds are especially rich in gibberellins (10-100 mg per g fresh weight) and are most favorite plant parts for isolation of gibberellins and their study. In mature seeds, the gibberellins tend to form their derivatives.

In plant, the gibberellins may occur in two different forms- free gibberellins and bound gibberellins. Bound gibberellins usually occur as gibberellins-glycosides.

Biosynthesis of gibberellins in plants

The gibberellins which are chemically related to terpenoids (natural rubber, carotenoids & steroids) are thought to be formed by the condensation of a 5-C precursor- an isoprenoid unit called as ispentenyl pyrophosphate (IPP) through a number of intermediates to give rise to gibberellins. The primary precursor for the formation of this isprenoid unit and synthesis of gibberellins is however, acetate. Besides gibberellins, carotenoids, rubber, steroids, abscisic acid (ABA) and part of cytokinins are also derived from 5-C isoprenoid unit.

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.

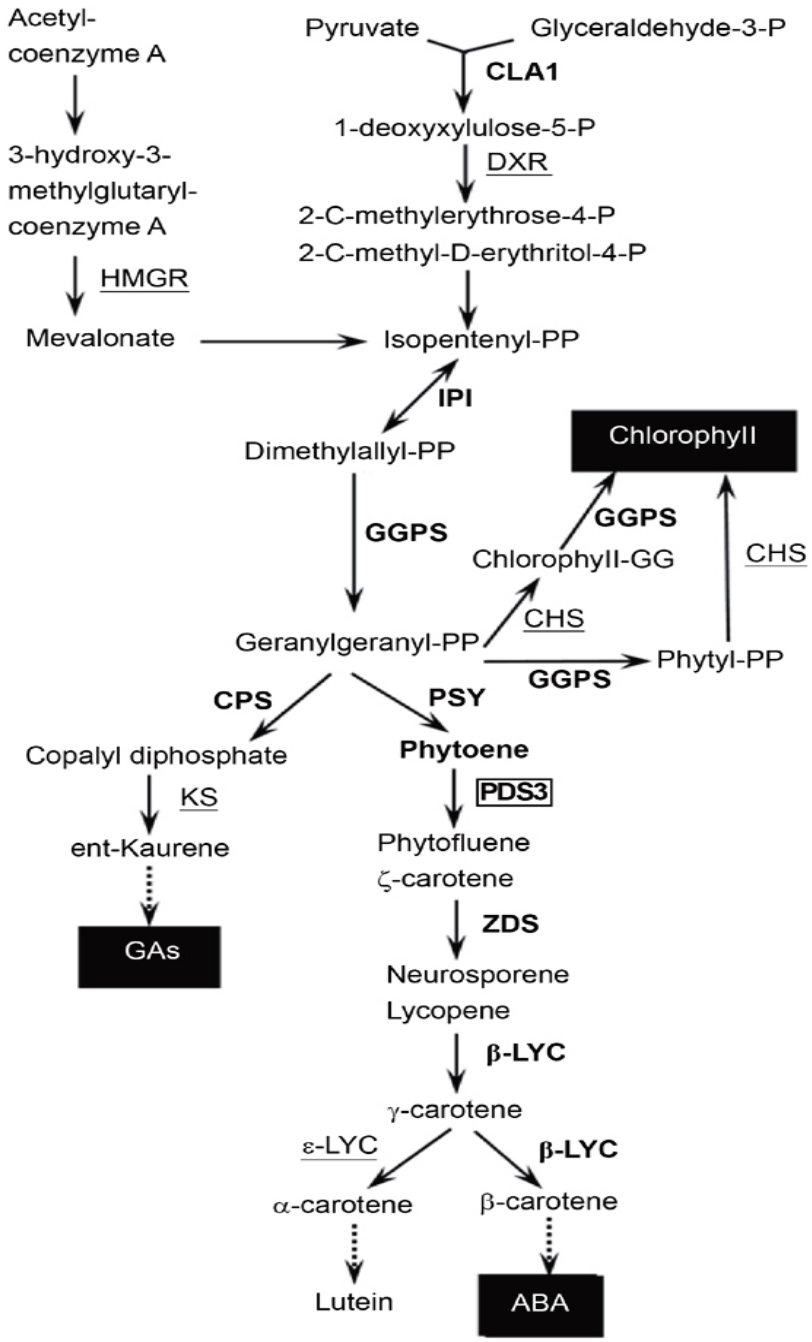
Stage II. Oxidations to form GA_{12} and GA_{53} on ER through GA_{12} aldehyde.

The **ent –kaurene** is transported from plastids to **ER** (**endoplasmic reticulum**). Now a methyl group on ent-kaurene at 19^{th}-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 **GA_{12} aldehyde**. GA_{12} aldehyde is subsequently oxidized to give **GA_{12} which is precursor to all other GAs in plants**. Hydroxylation of GA_{12} at C-13 results in the formation of GA_{53}.

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 GA_{12} –aldehyde.

Stage III. Formation of all other GAs from GA_{12} or GA_{53} in cytosol.

All other steps in the biosynthesis of GAs from GA_{12} of Ga_{53} are carried out in cytosol by soluble enzymes called dioxygenases. These enzymes require molecular O_2 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

http://www.nature.com/cr/journal/v17/n5/images/cr200740f1.jpg

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 plant as free molecules but probably in their bound form as gibberellins-glycosides.

Cytokinins

Discovery and chemical nature

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. They added various growth substances, nutrients, vitamins etc, into the culture medium but failed till they noticed an old bottle of DNA kept for several years in their lab. They added the contents of that bottle to the culture medium and observed that the tobacco pith callus could grow for longer periods. They obtained similar results with Yeast extract. But they did not get positive results with fresh DNA and thought the active substance to be some degradation product of DNA. They isolated this substance by autoclaving (heating under pressure) the DNA which had been stored for long. It could easily be precipitated by silver salts and was soluble in 90% alcohol, indicating that possibly it was a purine compound. Later on, they identified it as 6-furfurylaminopurine. Because of its specific effect on cytokinesis (*i.e*.,) cell division, it was called as kinetin.

Although kinetin has profound influences in inducing cell division, still it has not been isolated from any plant. But, certain substances which show kinetin like activity have in fact been isolated from a variety of higher plants. These substances are collectively called as cytokinins. There is now sufficient evidence to show that cytokinins do occur in plants and regulate growth and hence, they are also considered as natural plant growth hormones. Some of the very important and commonly known naturally occurring cytokinins are as follows.

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.

Other natural cytokinins

- Apart from zeatin, some other substituted amino purines have been isolated from higher plants and some bacteria which are also considered as natural cytokinins. These are di-hydrozeatin (DZ) and N6-(Δ2 - isopentenyl) adenine (or ip) which differ from Zeatin in nature of their side chain.

Cytokinins in t-RNA

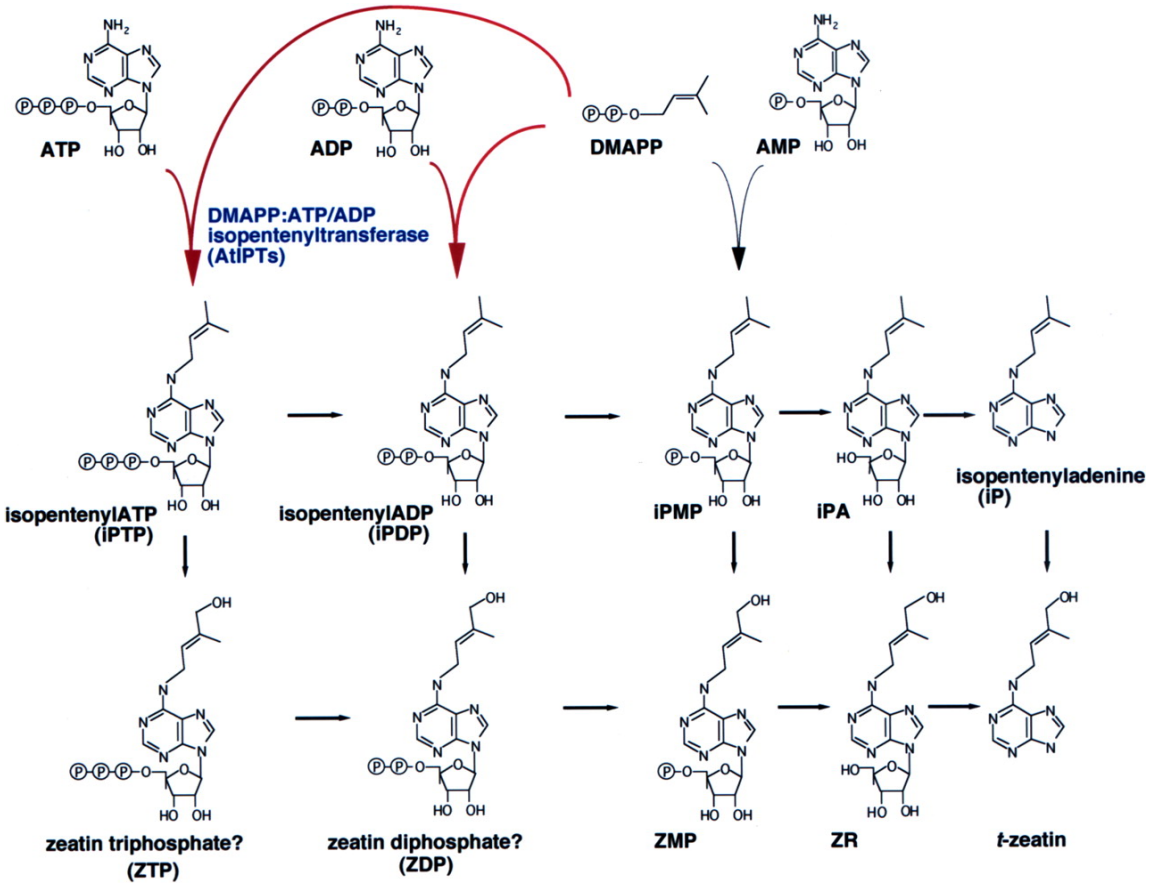
In 1966 Zachau *et al*. identified cytokinin 2iPA as a constituent of two serine t-RNA species from brewers yeast and showed this cytokinin to be adjacent to the 3' end of the anticodon in both the species. Apart from yeast, cytokinins have now been found in t-RNA preparations from a wide variety of organisms such as bacteria including *E.coli*, animals including man and higher plants *viz*., frozen peas, tobacco callus tissue.

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.

Biosynthesis of cytokinins



A model for cytokinin biosynthesis in plants. Cytokinins that directly bind to cytokinin receptors are *shaded*

http://pcp.oxfordjournals.org/content/42/7/677/F6.expansion.html

Physiological effects of kinetin (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. The inhibitory effect of far-red light treatment on the germination of the above seeds is also overcome by kinetin treatment.

7. Delay of senescence: The Richmond –Lang Effect

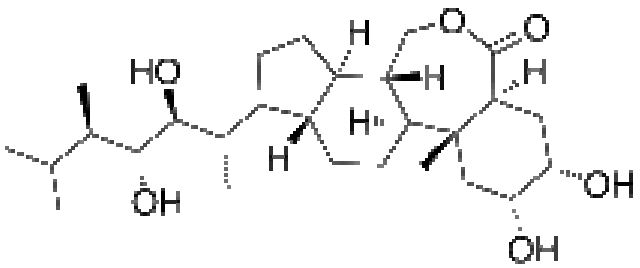
The ageing process of the leaves usually accompanies with loss of chlorophyll and rapid breakdown of proteins. This is called senescence. Richmond and Lang showed that this senescence could be postponed to several days in detached Xanthium leaves by kinetin treatment. This effect of kinetin in delaying the senescence is called as Richmond-Lang effect. One of the important factors in delay of senescence in kinetin treated leaves is their physiological age. Mature leaves of ***Nicotiana rustica*** have been found to be more responsive to kinetin treatment in delaying senescence than the younger leaves.

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. The yield of brassinosteriods from 230 kg of *Brassica napus* pollen was only 10 mg. Since their discovery, over 70 BR compounds have been isolated from plants.



Brassinolide

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.
- Is necessary for pollen elongation for pollen tube formation.
- Acceleration of senescence in dying tissue cultured cells; delayed senescence in BR mutants supports that this action may be biologically relevant.
- Can provide some protection to plants during chilling and drought stress.

Extract from the plant *Lychnis viscaria* contains a relatively high amount of Brassinosteroids. *Lychnis viscaria* is said to increase the disease resistance of surrounding plants. In Germany, extract from the plant is allowed for use as a "plant strengthening substance."

24-Epibrassinolide (EBL), a brassinosteroid isolated from *Aegle marmelos* Correa (Rutaceae), was further evaluated for the antigenotoxicity against maleic hydrazide (MH)-induced genotoxicity in *Allium cepa* chromosomal aberration assay. It was shown that the percentage of chromosomal aberrations induced by maleic hydrazide (0.01%) declined significantly with 24-epibrassinolide treatment.

BRs have been reported to counteract both abiotic and biotic stress in plants. 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.

Table 1. Physiological effects of brassinosteroids in plants.

Cell level	Whole plant level
Stimulation of elongation and fission	Growth promotion
Effect on hormonal balance	Increase in the success of fertilization
Effect on enzyme activity; H.-pump activation	Shortening the period of vegetative growth
Activation of protein and nucleic acid synthesis	Size and quantity of fruits increase
Effect on the protein spectrum and on the amino acid composition of proteins	Effect on the content of nutritive components and fruit quality improvement
Effect on the fatty acid composition and on the properties of membrane	Increased resistance to unfavourable environmental factors, stress and diseases
Enhancement of the photosynthetic capacity and of translocation of products	Crop yield increase

VLADIMIR Khripach *et al*. (2000)

Questions

1. The true natural auxin of higher plants is,

a. Indole-3-acetic acid b. Indole-3-acetaldehyde

c. Indole-3-pyruvic acid d. Indole-3-acetonitrile

Ans: a. indole-3-acetic acid

2. The primary physiological effect of auxin in plants is,

a. Elongation of internodes b. Cell division

c. Elongation of cells in shoot d. None of the above

Ans: b. Cell division

3. Auxin is synthesized mainly in,

a. Roots b. Meristermatic regions of the plant

c.Shoots d. None of the above

Ans: b. meristermatic regions of the plant

4. The precursor for the synthesis of auxin in plants is

a. Tryptophan b. Tyrosine c. Proline d. None of the above

Ans: a. Tryptophan

5. Transport of auxin in plant is predominantly,

a. Polar b. Non-polar c. Lateral d. None of the above

Ans: a. Polar

6. Gibberellins are derivatives of

a. Monoterpenes b.Sesquiterpenes c. Diterpenes d. Triterpenes

Ans: c. Diterpenes

7. Which of the following is not a specific physiological effect of gibberllins?

a. Elongation of internodes b. bolting and flowering

c. De novo synthesis of α-amylase d. cell division

Ans: d. cell division

8. Richest source of gibberellin in higher plant is

a. Root b. Stem c. Leaf d. Immature seeds

Ans: d. immature seeds

9. Gibberellin transport in plant is predominantly

a.Polar b. Lateral c. Non-polar d. None of the above

Ans: c. Non-polar

10. The credit for discovery of kinetin goes to

a. Miller *et al* b. Letharn *et al* c. Zachau *et al* d. none of the above

Ans: a. Miller *et al.*

11. Chemical name of kinetin is,

a. 6-furfuryl amino purine b. 6-furfurylamino pyrimidine

c. 5-furfurylamino purine d. None of the above

Ans: a. 6-furfuryl amino purine

12. Most important biological effect of kinetin in plants is to induce,

a. Cell enlargement b. Cell division

c. Elongation of internodes d. None of the above

Ans: b. Cell division

13. Fruit ripening hormone is,

a. ethylene b. auxin c. kinetin d. All of above

Ans: a. ethylene

14. 'Triple response' of etiolated pea seedlings is caused by,

a. ABA b. IAA c. Ethylene d. None of the above

Ans: c. Ethylene

15. Brassinosteroids were first isolated from honey bee collected pollen grains of,

a. *Brassica compestris* b. *B. napus* c. *B. oleracea* d. All of above

Ans: b. *B.napus*

16. Brassinosteroids are biosynthesized in plants from

a. Sitosterol b. Cholesterol c. Campesterol d. None of the above.

Ans: c. Campesterol

Lecture No.5

Abscisic acid, Ethylene and other growth retardants

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.

Warning *et al.* (1963, 64) pointed out the presence of a substance in birch leaves (*Betula pubescens*, a deciduous plant) which inhibited growth and induced dormancy of buds and, therefore, named it **'dormin'**. But, very soon as a result of the work of **Cornforth *et al* (1965)**, it was found to be identical with abscisic acid.

Abscisic acid is a 15-C **sesquiterpene** compound (molecular formula $C_{15}H_{12}O_4$) composed of three isoprene residues and having a **cyclohexane ring** with **keto** and one **hydroxyl** group and a side chain with a **terminal carboxylic group** in its structure.ABA resembles terminal portion of some carotenoids such as violaxanthin and **neoxanthin** and appears to be a breakdown product of such carotenoids.Any change in its molecular structure results in loss of activity. ABA occurs in *cis* and *trans* isomeric forms that are decided by orientation of –COOH group around 2^{nd} carbon atom in the molecule. Almost **all naturally occurring ABA** in plants exist **in cis form** that is **biologically active** and the name abscisic usually refers to this form. Trans-ABA is **inactive** form but can be interconvertible with *cis* ABA.

Physiological role

1. Stomatal regulation

The role of ABA in causing stomatal closure in plants undergoing water-stress is now widely recognized. It has been suggested by various workers that in response to the water-stress, the permeability of the chloroplast membranes of mesophyll cells to ABA is greatly increased. As a result, the ABA synthesized and stored in mesophyll chloroplasts diffuses out into the cytoplasm. It then moves from one mesophyll cell to another through plasmodesmata and finally reaches the guard cells where it causes closing of stomata. Fresh biosynthesis of

ABA continues in mesophyll chloroplasts during periods of water stress. When water potential of the plant is restored (*i.e*., increased), the movement of ABA into the guard cells is arrested. ABA disappears from the guard cells a little later. 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 α-amylase in aleurone layers of germinating barley, inhibition of precocious germination and vivipary and increase in root: shoot ratio at low water potentials.

Biosynthesis of ABA in plants

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.**

Occurrence and distribution of ABA in plants

Within the plant, ABA has been detected in all major organs or living tissues from root caps to apical buds such as roots, stems, buds, leaves, fruits and seeds and also in phloem and xylem sap and in nectar. ABA is synthesized in all types of cells that contain chloroplasts or other plastids. It occurs predominantly in mature green leaves. Most plant tissues contain ABA in concentration of 20-100 ng per g fresh weight, but higher conc. of 10 μg and 20 μg

per g fresh weight have been reported in avocado fruit pulp and dormant buds of cocklebur (*Xanthium* spp.) respectively. The concentration of ABA in specific plant tissues varies greatly at different developmental stages or in response to environmental conditions especially water stress. For instance, in developing seeds ABA conc. may increase 100 fold within a few days and decline as the seed matures. Similarly, under water stressed conditions, ABA level may increase 50 fold in the leaves within a few hours and declines to normal when plant water potential is restored. 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-β-D-glucosyl ester. ABA is biologically inactive in its conjugated or bound form.

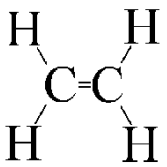
Ethylene

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. These symptoms were later termed as **'triple response'** and were not observed in etiolated pea seedlings grown in normal air free from coal gas.

Gane (1934) clearly established that ethylene is actually a natural product of ripening fruits and is responsible for hastening ripening process. Meanwhile, several other experimenters found evidence of ethylene being produced not only by ripening fruits but also by flowers, seeds, leaves and even roots and having profound regulatory activity in plants. The importance of ethylene as hormonal regulator of physiological processes was realized only after the **advent of gas chromatography (GC)** and its use in ethylene research, (Burg and Thimann, 1959, 60). Soon, this was followed by an avalanche of experimental research work on ethylene and **finally ethylene emerged as an accepted natural plant growth hormone (Pratt and Goeschal, 1969).**

Chemical nature

Ethylene (C_2H_4) with a molecule weight of 28 is a well known and simplest olefin gas and has the following structural formula.



Ethylene is flammable and highly voltaic substance that readily undergoes oxidation to produce ethylene oxide. In many plant tissues, ethylene can be fully oxidized to CO_2 through ethylene oxide. It is colourless, lighter than air at room temperature and under physiological conditions and is sparingly soluble in water. Ethylene is readily absorbed by potassium permanganate ($KMnO_4$). The latter is frequently used to remove excess ethylene from the storage changers.

Physiological role

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**.

Different types of fruits react differently with exogenous application of ethylene. In **climacteric** fruits such as apples, bananas, tomatoes etc., exposure of mature fruits to ethylene result in **respiration climacteric** (marked increase in respiration rate during initiation of ripening) followed by **additional production of ethylene** leading to **hastening of ripening process**. Additional production of ethylene by ripening fruits is **autocatalytic**. But, in non-climacteric fruits such as citrus fruits and grapes, ethylene treatment does not cause respiration climacteric and additional ethylene production and the rate of ripening process remains unaffected.

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.

The plumular **hook formation** and its **maintenance** in etiolated (dark grown) seedling are due to formation of **ethylene** in that region which causes **asymmetric or unequal growth** on the two sides of plumular tip. Ethylene causes more rapid elongation of outer side of plumular tip than on its inner side. When the seedling is exposed to **white light**, formation of ethylene decreases, the inner side of the hook also elongates rapidly equalizing the growth on two sides and the hook opens.

Red light is more effective in **opening** of plumular **hook**. This effect is reversed by **exposing the seedling to far-red- light**. This red/far-red reversibility is indicative of the role of the pigment phytochrome in it.

When etiolated seedlings are **exposed to light in presence of ethylene**, the plumular **hook fails to open**. On the other hand, if seedlings are grown in dark along with an ethylene absorbent such as $\mathbf{KM_nO_4}$ the plumular **hook opens**.

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. Young leaves are more sensitive than the older leaves. However, **monocots do not exhibit this response**.

Higher concentration of auxin, stress conditions such as salt stress, water-logging and pathogen infection also induce leaf epinasty **indirectly** through increased ethylene formation. In tomato and other plants, water-logging creates **anaerobic condition** around the roots resulting in **accumulation of ACC** (**1-amino cyclopropane-1-carboxylic acid**) (the immediate precursor of ethylene formation) in roots. ACC is then translocated to shoots along with transpiration stream where it is converted into ethylene in presence of oxygen and induces leaf epinasty.

7. Flowering

Ethylene is known to inhibit flowering in plants. However, in pineapple and its allies (Family Bromeliaceae) and also in mango, it induces flowering. Ethylene is used commercially to synchronize flowering and fruit set in pineapple.

Plumbogo indica (Short Day Plant) can be made to flower even under non-inductive long days with the application of ethylene.

8. Sex Expression

In monoecious species (with separate male and female flowers on the same plant) 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.

Freshly cut carnation flowers when held in water in a conical flask, loose colour of their petals and wither (*i.e.*, senescence) within a few days. But, if the cut carnations are held in conical flask containing **silver thiosulphate solution**, they remain fresh for many weeks. This

is because silver **thiosulphate is potent inhibitior of ethylene action**. Role of ethylene in enhancing senescence has now been confirmed by studies with transgenic plants also.

10. Abscission of leaves

Ethylene **promotes abscission of leaves** in plants. Older leaves are more sensitive than the younger ones. Fumigating the wild type birch tree (***Betula pendula***) with 50 ppm ethylene results in rapid defoliation of the tree within few days.

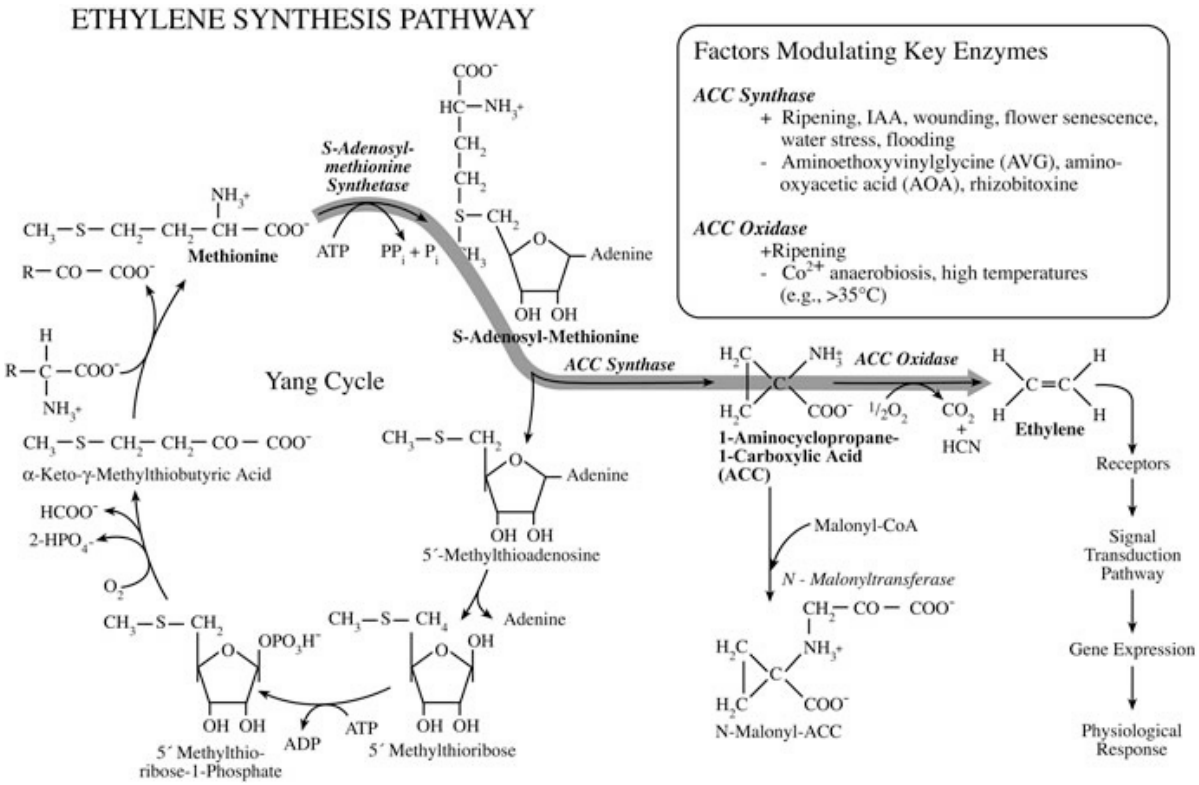
The relative concentration of auxin on two sides of the abscission layer has regulatory influence on the production of ethylene that stimulates leaf abscission. At the time of abscission, concentration of auxin in laminar region decreases with simultaneous increase in ethylene production. This also **increases sensitivity of cells of abscission zone to ethylene** which now synthesize cell wall degrading enzymes such as cellulases and pectinases. Activity of these 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. Non-dormant varieties of seeds produce more ethylene than those of dormant varieties.

In many plants, rate of seed germination is increased by ethylene and a close correlation has been found between ethylene formation and seed germination in peanuts (*Arachis hypogaea*). In many plants, **dormancy of buds** can also be broken by ethylene treatment. Sometimes, potato tubers are exposed to ethylene in order to **sprout the dormant buds.**

Biosynthesis of ethylene



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Ethylene is known to be synthesized in plant tissues from the amino acid **methionine**. A non-protein amino acid, **ACC** is an important intermediate and also **immediate precursor** of ethylene biosynthesis. The two carbons of ethylene molecule are derived from carbon no.3 and 4 of **methionine**. Whole process of ethylene bio-synthesis is a three steps pathway and is aerobic:

- **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 adenosyl transferase).

- **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.

- **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 H_2O 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.

Factors Stimulating Ethylene Biosynthesis

Ethylene biosynthesis is known to be stimulated by a number of factors such as **IAA, cytokinins, fruit ripening, stress conditions** (drought, flooding, chilling exposure to ozone etc.) and **mechanical wounding**. In all these cases, ethylene biosynthesis is stimulated by **induction of ACC synthase.** In **climacteric fruits**, ethylene itself promotes biosynthesis of ethylene by **autocatalysis.**

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

Role

- 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

- Jasmonic acid (JA), is a cyclopentanone derivatives synthesized from linolenic acid via the octadecanoic pathway. 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-induced-proteins** 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 leaves of chlorophyll-deficient *Hordeum vulgare* mutants.
- They exist in etiolated leaves, though Jasmonates do not only regulate the transcription of these proteins, they do also influence the rate of translation of different groups of mRNA.
- They do, for example, decrease the production rate of several essential housekeeping proteins. Just like ABA, jasmonates also inhibit premature germination of the oil-containing seeds of *Brassica* and *Linum*. After germination, they do induce the synthesis of the seed storage proteins Napin and Cruciferin as well as that of several more elaiosome-associated proteins.

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^{-3}M) of applied methyl jasmonate. Skin colour of ripe fruit was significantly improved with exogenous application of methyl jasmonate (10^{-3}M). Methyl jasmonate treatments also increased the concentration of fatty acids as well as total aroma volatiles, monoterpenes, sesquiterpenes, aromatics,

norisoprenoid, alcohols and esters in the pulp of fruit. However, exogenous application of methyl jasmonate tended to reduce production of n-tetradecane, especially on day 5 and 7 of ripening. In general, exogenous application of methyl jasmonate (10^{-3}M) significantly promoted biosynthesis of ethylene, fatty acids and ripening and aroma volatile compounds during fruit ripening. Our experimental results suggest that methyl jasmonte is involved in early steps in the modulation of mango fruit ripening (Lalel *et al*., 2003).

- Raspberries treated with MJ had higher soluble solids content, total sugars, fructose, glucose, sucrose and lower titratable acids (TAs), malic acid and citric acid 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
- When gibberellin production is inhibited, cell division still occurs, but the new cells do not elongate. The result is shoots with the same numbers of leaves and internodes compressed into a shorter length.
- Recent research has demonstrated that blocking a portion of the so-called terpenoid pathway causes shunting of the accumulated intermediary compounds above the blockage. The consequence is increased production of the hormone abscisic acid and the chlorophyll component phytyl, both beneficial to tree growth and health
- Paclobutrazol treated trees have greater tolerance to environmental stresses and resistance to fungal diseases. Morphological modifications of leaves induced by

treatment with paclobutrazol such as smaller stomatal pores, thicker leaves, and increased number and size of surface appendages on leaves may provide physical barriers to some fungal, bacterial and insect infections.

Influence in fruit culture

- Treated trees of apple have more compact crowns and somewhat 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. As a consequence of the reduced growth in height, there is a parallel reduction in biomass removed when trees eventually require trimming
- Increased concentration of paclobutrazol (1000ppm) 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 2 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 color suggesting high chlorophyll content. There are two possible explanations for this response. One is that the leaves of both treated and untreated trees contain the same number of cells, but because the cells in leaves of treated trees are smaller, the chlorophyll is more concentrated in the reduced cell volume. 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.

Growth regulators are mainly used as foliar spray, dipping cuttings and fruit bunches (grapes) and soil application is not commonly followed.

Questions

1. Which of the following is considered as naturally occurring growth inhibitor in plants?

a. IAA b. ABA c.GA_1 d. all of above

Ans: b. ABA

2. Abscisic acid (ABA) is not involved in,

a. Stomatal closure in water stressed plants

b. Fruit ripening and dormancy of seeds and buds

c. Senescence

d. Cell elongation

Ans: d. Cell elongation

3. Which of the following is not an antigibberellin or growth retardant?

a. Phosfon D b. Cycocel c. Amo-1618 d. Benzyl adenine

Ans: d. Benzyl adenine

4. Antigibberellins such as cycocel (CCC) find extensive use in

a. food industry b. breweries c. floriculture industry d. none of the above

Ans: c. floriculture industry

5. Minimum lag time for auxin-induced growth is,

a. 10 minutes b. 15 minutes c. 20 minutes d. 30 minutes

Ans: a. 10 minutes

6. Most frequently occurring organic acids in fruit cells are,

a. malic acid b. citric acid c. both a and b d. tartaric acid

Ans: c. both a and b

7. Immediate precursor of ethylene biosynthesis in plants is,

a. Methionine

b. S-Adenosyl methionine

c. 1-Aminocyclopropane-1-carboxylic acid (ACC)

d. None of these

Ans: c. 1-Aminocyclopropane-1-carboxylic acid (ACC)

8. Which one of the following is not an inhibitor of ethylene biosynthesis?

a. AVG b.AOA c. Co^{2+} d. None of these

Ans: d. None of these

9. A potent inhibitor of ethylene action is,

a. silver ions b. Cobalt ions c. Manganese ions d. None of these

Ans: a. silver ions

10. ABA is a,

a. Sesquiterpene b. Diterpene c. Triterpene d. Tetraterpene

Ans: a. Sesquiterpene

11. Naturally occurring, biologically active ABA in plants exists in,

a. Trans form b. Cis form c. Both a & b d. None of these

Ans: b. Cis form

12. The primary hormones causing abscission of leaves is,

a. ABA b. IAA c. Ethylene d. Cytokinin

Ans: a. ABA

13. ABA is synthesized in plants,

a. directly from simple terpenoid precursors

b. indirectly through carotenoid breakdown

c. through shikimic acid pathway

d. none of these

Ans: b. indirectly through carotenoid breakdown

14. ABA is inactivated in plant cells by converting into,

a. phaseic acid b. dihydrophaseic acid

c. ABA-glycosyl ester d. all of these

Ans: d. all of these

15. ABA occurs in plants predominantly in,

a. Roots b. Stems c. Mature green leaves d. Flowers

Ans: c. Mature green leaves

Lecture No.6

Mechanisms of Abscission and Senescence of leaves

Abscission of leaves

Detachment of the older (rather senescent) leaves or leaf fall is a common phenomenon in plants and is called as abscission of leaves. Abscission is quite distinctive in deciduous trees and shrubs of temperate regions in autumn when all the leaves of such plants fall at about the same time giving the plants a naked appearance, the new leaves developing in the subsequent spring. In evergreen plants there is gradual abscission of leaves, the older leaves fall while new leaves are developed continuously throughout the year. In most of the herbaceous species, however the leaves are not shed even after they die and in many cases are retained in withered dry condition even after the whole shoot is dead. Leaf abscission takes place at the base of the petiole which is internally marked by a distinct zone of few layers of thin-walled cells arranged transversally across the petiolar base. This zone is called as the abscission zone or abscission layer. The cells of the abscission layer separate from each other due to the dissolution.

Mechanism of abscission:

The young leaves remain attached to the stem and do not abscise till they become old. However, if the blade or lamina portion of a young leaf is cut, the debladed petiolar stump soon abscises. In case auxin (IAA) in lanolin paste is applied to the cut end of petiole of such a young leaf the abscission of the petiolar stump is greatly suppressed. The intact young leaf does not abscise because its lamina portion contains auxin synthesized by it. These experiments have led to the belief that auxin has controlling influence in the abscission of leaves. This belief is further strengthened by the fact that endogenous auxin concentration in leaves falls considerably at the time of normal abscission.

In yet another experiment, it has been found that if auxin is applied to the distal side (blade side) of the abscission zone of the debladed petiole of young leaf, the abscission of the petiolar stump is prevented. On the other hand, if the auxin is applied to the proximal side (stem side) of the abscission zone of the debladed petiole, abscission is accelerated.

Normally, the auxin level of the stem side of the abscission zone is probably maintained due to basipetal transport of auxin from the stem tip while the source of the auxin on the blade side of the abscission zone is the blade or lamina of leaf itself. The above-mentioned experiments have led to the establishment of auxin gradient hypothesis according to which it is not the

presence or absence of auxin but relative concentration of auxin on stem side of the abscission zone or nearly equal concentration of auxin on both its sides will promote abscission while higher concentration of auxin on the blade side of the abscission zone will retard abscission.

The mechanism by which auxin controls abscission is not clearly understood. Besides auxin, other growth hormones especially ethylene may also play important role in abscission.

Recent researches have now shown that the **relative concentration of auxin** on two sides of the abscission layer has **regulatory influence** on the production of ethylene that stimulates leaf abscission. At the time of abscission, concentration of **auxin** in the laminar region decreases with simultaneous **increase in ethylene production.** This also **increases sensitivity of cells of abscission zone to ethylene** which now synthesize cell wall degrading enzymes such as **cellulases** and **pectinases.** Activity of these enzymes results in cell wall loosening and cells separation ultimately leading to leaf abscission.

Senescence in plants

The plants or their organs like all other living organisms have a certain span of life during which they develop, grow, attain maturity and after some time die. But prior to death, distinctive but natural **deteriorative processes** that naturally terminate their functional life are collectively called as senescence and the plants or plant organs at this stage are called as senescent. These deteriorative processes may terminate in death either gradually or abruptly depending upon the plant. **Senescence** is a **normal energy dependent developmental process** which is **controlled by** plant's **own genetic programme** and the death of the plant or plant part consequent to senescence is called as **programmed cell death (PCD).**

Senescence is not confined only to whole plant. It may be limited to a particular plant organ such as leaf and flowers or cells such as phloem and xylem or cell-organelles such as chloroplasts and mitochondria etc.

Senescence is closely associated with the phenomenon of aging and both are sometimes considered as the same by many workers. But according to Medawar (1957), the term senescence should be used to refer to natural changes towards termination of life while aging to refer to changes in time without reference to the natural development of death.

Leopold (1961) has recognized 4 types of senescence patterns in whole plant which are as follows:

1. **Overall Senescence**. This type of senescence occurs in annuals where whole of the plant is affected and dies.
2. **Top Senescence**. This is represented by perennial herbs where senescence occurs only in the above ground parts, the root system and underground system remaining viable.
3. **Deciduous Senescence**. This type of senescence is less drastic and takes place in woody deciduous plants. Here senescence occurs in all the leaves simultaneously but the bulk of the stem and root system remains alive.
4. **Progressive Senescence**. This is characterized by gradual progression of senescence and death of leaves from the base upwards as the plant grows.
   (The senescence of the entire plant after a single reproductive cycle is also known as **monocarpic senescence**)

Senescence can best be studied in leaves or similar other organs of plants e.g. cotyledons, sepals, petals etc. or cell organelles like isolated chloroplasts.

- Senescing cells and tissues are metabolically very active and an ordered series of cytological and biochemical events occur during senescence.
- Senescence is characterized by increased respiration, declining photosynthesis and an orderly disintegration of macromolecules.
- At the cellular level, chloroplasts are the first organelles to be disintegrated. Nuclei remain structurally and functionally intact until the last stage of senescence. Meanwhile, other cell organelles and biomembranes also gradually deteriorate.
- **Expression of senescence down-regulated genes (SDGs) decreases**. Such genes encode proteins in photosynthesis and other biosynthetic processes. Concentration of growth promoting hormones especially **cytokines** declines.
- **Expression of senescence associated genes (SAGs) increase**. Such genes encode **hydrolytic enzymes** such as **proteases**, **ribonucleases** and **lipases** as well as enzymes involved in biosynthesis of deteriorative hormones such as **abscisic** acid **(ABA)** and **ethylene.**
- Some of the SAGs have secondary functions in senescence that are useful to plant. These genes encode enzymes that are involved in **conversion and**

remobilization of nutrients and substrates from senescing tissues and their **reallocation** to other parts of the plant that survive (i.e., not senescing).

- **Brilliant Colours** are developed in leaves of many plants during senescence.
- Towards the end of senescence, the cells and tissues also lose respiratory control.

Several environmental factors especially those which suppress normal plant growth also tend to enhance the rate of senescence. These are deficiency of soil nutrients, high temperatures, water deficit, darkness etc.

In many plants on the other hand, removal of flowers, fruits and vegetative growing points can markedly delay the senescence of leaves. The role of externally supplied cytokinins in delaying senescence especially in detached plant part is also well established.

Programmed Cell Death (PCD)

As mentioned earlier, senescence is controlled by plants own genetic programme and the death of the plant or plant part consequent to senescence is called as **programmed call death (PCD).** The distinct set of morphological and biochemical changes accompanying the PCD have also been called as **apoptosis** (from a Greek Word meaning 'falling off').

PCD plays an important role in normal vegetative and reproductive development in plant and also in defense against pathogens:

- Senescence is one form of PCD. The nutrients and other substrates from senescing cells and tissues are remobilized and reallocated to other parts of the plant that survive.
- The protoplasts of developing tracheary elements (xylem vessels and tracheids) die and disappear at maturity to make elements functionally efficient as conduit for water transport.
- In aquatic plants, aerenchyma is normally formed in different parts of the plant such as roots and stems which enclose large air spaces that are created via PCD.
- In the development of unisexual flowers, primordial for both male and female flowers are present in the earlier stages, but only one of these two completes its development while the other aborts via PCD.

- In ovules, the megaspore mother cell divides meiotically to form four megaspores of which only one remains functional to form the female gametophyte while the rest degenerate. This is predetermined and genetically controlled and is an example of programmed cell death.
- On being infected by a pathogen, the host plant cells die rapidly around the infection site forming a necrotic lesion. This deprives the pathogen of the nutrient supply and prevents its spread in the host plant. This is also a form of PCD that is beneficial to plant.

Lecture No. 7

Flowering: Floral development-factors affecting flowering

Initiation of flower primordial

The initiation of flower primordia is a major event in the life cycle of a plant in that it involves a shift in the phase of development from vegetative to reproductive processes. The significance of flower initiation has been recognized by botanists for many years. In 1918, Klebs suggested that during the life cycle of plant it passes through several phases of development. Before floral primordia can be initiated, the plant must complete a period of vegetative growth or attain some minimal leaf number. When this condition is attained, the plant is said to be ripe to flower. Ripeness-to-flower is not recognized by any external characteristics, but it can be determined empirically by subjecting plants of varying age (From seedling emergence) to environmental conditions known to induce flowering. In most plants ripeness – to- flower is attained after the plant has produced several leaves. In some cereal grasses a minimum of seven leaves must be developed before the plant is ripe to flower. On the other hand, a plant like Pharbitis nil, the Japanese morning glory, is ripe to flower within a day after the cotyledons have emerged. Cotyledons presumably contain enough stored food to support subsequent reproductive development. Most common cultivated plants and weedy annuals, however, attain the ripe-to-flower condition 2 to 3 weeks after seedling emergence when a few leaves have fully developed.

Attainment of the ripe-to-flower condition does not automatically lead to the initiation of flower primordial. Certain environmental conditions must follow. These same environmental conditions, if presented to a plant that is not ripe to flower, elicit no flowering response. The importance of temperature and the promotion of reproductive development, a phenomenon referred to as vernalization, were described by Gassner in 1918. About the same time W.W Garner and H. A. Allard (1920) two plant physiologists with the U.S. Department of Agriculture, found that day length, or the duration of light and dark periods within a 24 hour cycle, also influenced the initiation of flowering.

Since the early work of Klebs, Gassner, and Garner and Allard, plant physiologists have carried out detailed studies of the anatomical, chemical, and biochemical processes that accompany the shift from vegetative to reproductive growth. Although much is still not known

about floral initiation, several different kinds of processes are thought the choice of appropriate plant material and experimental techniques, to isolate individual reactions for detailed study. The transport of the flower stimulus or the transformation of the shoot apex can be studied, for example. In certain plants it has been possible to determine the length of time necessary to complete some of the partial reaction.

Other aspects of reproductive development:

The change from vegetative to reproductive growth not only involves switching the shoot apex from leaf formation to flower formation

Lecture No.8

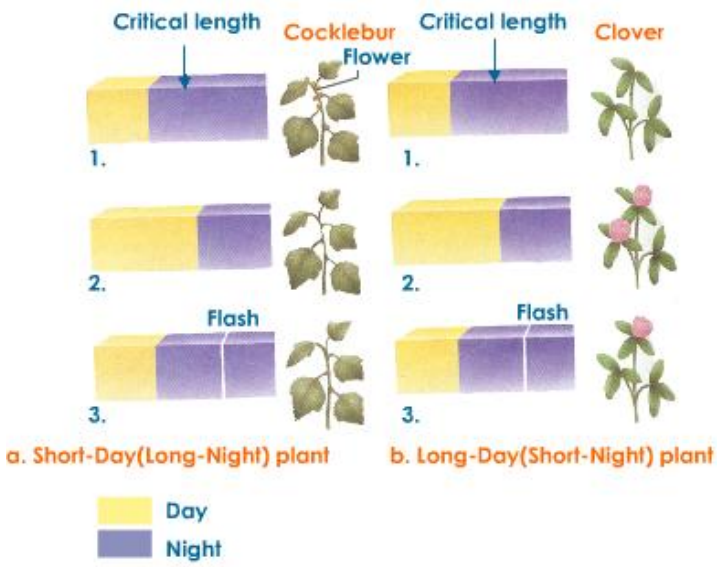
Physiology of Flowering in plants

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.



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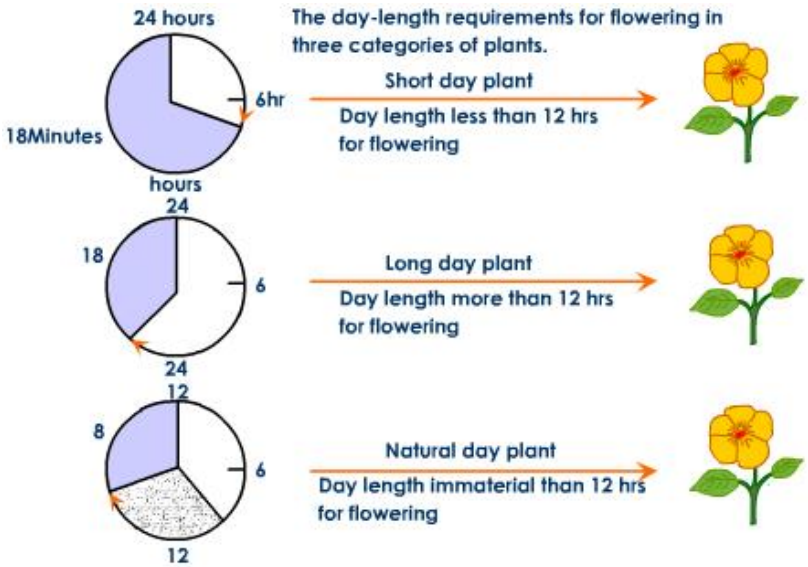
Short Day Plants (SDP)

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).*



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The Day-length Requirements for Flowering in Three Categories of Plants

- In short day plants the dark period is critical and must be continuous. If this dark period is interrupted even with a brief exposure of red light (660-665 mμ (μm = 10^{-6} mm) wavelength), the short day plant will not flower.

Short Day Plant

16 hours dark	8 hours light

A. Short Day Plant flowers short day plant flowers

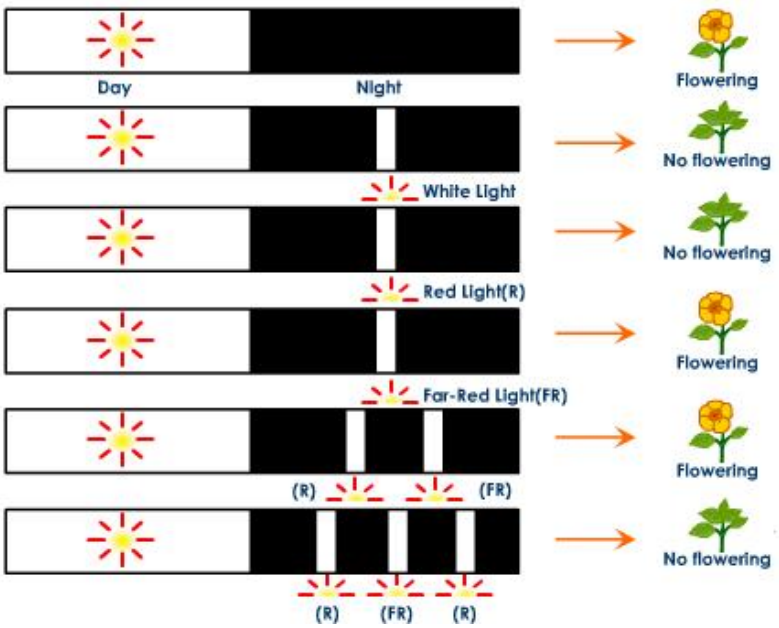
		DARK	LIGHT

B.Critical dark period. Short day plant does not flower interrupted by light

DARK			LIGHT

C. Light period interrupted by dark

- Maximum inhibition of flowering with red light occurs at about the middle of critical dark period.
- However, the inhibitory effect of red light can be overcome by a subsequent exposure with far-red light (730-735 mμ (μm = 10^{-6} mm) wavelength)
- Interruption of the light period with red light does not have inhibitory effect on flowering in short day plants.
- Prolongation of the continuous dark period initiates early flowering in short day plants.



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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).

- In long day plants the light period is critical
- A brief exposure in the dark period or the prolongation of the light period stimulates flowering in long day plants.

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. They are,

Long Short Day Plants

These are short day plants but must be exposed to long days during early periods of growth for subsequent flowering. Some of the examples of these plants are certain species of *Bryophyllum.*

Short-Long Day Plants

These are long day plants but must be exposed to short days during early periods of growth for subsequent flowering. Some of the examples of these plants are certain varieties of wheat (*Triticum*) and rye (*Secale*).

Lecture No.10

Photoperiodic Induction and Floral Hormone

Plants may require one or more **inductive cycles** for flowering. An appropriate photoperiod in 24 hours cycle constitutes one inductive cycle. If a plant which has received sufficient inductive cycles is subsequently placed under unfavorable photoperiods, it will still flower. Flowering will also occur if a plant receives inductive cycles after intervals of unfavorable photoperiods (*i.e*., discontinuous inductive cycles.) This persistence of photoperiodic after effect is called **photoperiodic induction**.

- An increase in the number of inductive cycles results in early flowering of the plant. For instance Xanthium (a short day plant) requires only one inductive cycle and normally flowers after about 64-days. It can be made to flower even after 13 days if it has received 4-8 inductive cycles. In such cases the number of flowers is also increased.
- Continuous inductive cycles promote early flowering than discontinuous inductive cycles. Some of the examples of plants which require more than one inductive cycle for subsequent flowering are Biloxi soybean (SDP) -2 inductive cycles; *Salvia occidentalis* (SDP)-17 inductive cycles; *Plantago lanceolata* (LDP) – 25 inductive cycles.

Perception of Photoperiodic Stimulus and Presence of a Floral Hormone

It is now well established that the photoperiodic stimulus is perceived by the leaves. As a result, a floral hormone is produced in the leaves which are then translocated to the apical tip, subsequently causing the initiation of floral primordial.

That the photoperiodic stimulus is perceived by the leaves can be shown by simple experiments on cocklebur *(Xanthium pennsylvanicum*), a short day plant. Cocklebur plant will flower if it has previously been kept under short-day conditions. If the plant is defoliated and then kept under short day condition, it will not flower. Flowering will also occur even if all the leaves of the plant except one leaf have been removed.

If a cocklebur plant whether intact or defoliated, is kept under long day conditions it will not flower. But, if even one of its leaves is exposed to short day condition and the rest are under long day photoperiods, flowering will occur.

The photoperiodic stimulus can be transmitted from one branch of the plant to another branch. For example, if in a two branched cocklebur plant one branch is exposed to short day

and other to long day photo period, flowering occurs on both the branches. Flowering also occurs if one branch is kept under long day conditions and other branch from which all the leaves except one have been removed is exposed to short day condition. However, if one branch is exposed to long photoperiod and the other has been removed is exposed to short day condition. However, if one branch is exposed to long photoperiod and the other has been defoliated under short day condition, flowering will not occur in any of the branches.

Nature of the Floral Hormone

The nature of floral hormone was named as florigin, which can be translocated from leaves to the apical tips situated at other parts of the plant resulting in flowering.

Grafting experiments in cocklebur plants have even proved that the floral hormone can be translocated from one plant to another. For example, if one branched cocklebur plant which has been exposed to short day conditions is grafted to another cocklebur plant kept under long day condition, flowering occurs on both the plants . Obviously the floral hormone has been transmitted to the receptor plant through graft union. But if a cocklebur plant is grafted to another similar plant both of which have been kept under long day conditions, flowering will not occur on either of the two plants.

It has also been indicated that the floral hormone may be identical in short-day and long-day plants. For example, grafting experiments between certain long-day plants and short –day plants have shown that flowering occurs on both the plants even if one of them has been kept under non-inductive photoperiods.

Phytochrome

It has already been seen that a brief exposure with red light during critical dark period inhibits flowering in short-day plants and this inhibitory effect can be reversed by a subsequent exposure with far-red light. Similarly, the prolongation of the critical light period or the interruption of the dark period stimulates flowering in long-day plants involves the operation of a proteinaceous pigment called as **phytochrome.**

- The pigment phytochrome exists in two different forms, (i) red light absorbing form which is designated as $\mathbf{P_R}$ and (ii) far-red absorbing form which is designated as $\mathbf{P_{FR}}$**.**

- When $\mathbf{P_R}$ form of the pigment absorbs red light (660-665 nm, it is converted into $\mathbf{P_{FR}}$ form).
- When $\mathbf{P_{FR}}$ form of the pigment absorbs far-red light (730-735 nm) converted in to $\mathbf{P_R}$ form.
- The $\mathbf{P_{FR}}$ form of the pigment gradually changes into $\mathbf{P_R}$ form in dark.

It is considered that during the day the $\mathbf{P_{FR}}$ form of the pigments is accumulated in the plant which is inhibitory to flowering in short-day plants but is stimulatory in long-day plants. During critical dark period in short-day plants, this form gradually changes into $\mathbf{P_R}$ form resulting in flowering. A brief exposure with red light will convert this form again into P_{FR} form thus inhibiting flowering. Reversal of the inhibitory effect of red light during critical dark period in SDP by subsequent far-red light exposure is because the $\mathbf{P_{FR}}$ form after absorbing far-red light (730-735 nm) will again be converted back into $\mathbf{P_R}$ form.

Prolongation of the critical light period or the interruption of the dark period by red light in long-day plants will result in further accumulation of the P_{FR} form of the pigment, thus stimulating flowering in long-day plants.

The phytochrome is a soluble protein with a molecular weight of about 250 kDa. It's a homodimer of two identical polypeptides each with a molecular weight of about 125 kDa. Each polypeptide has a prosthetic group called as chromophore which is covalently linked to the polypeptide via a sulphur atom in the cystine residue of the polypeptide. The protein part of the phytochrome is called as apoprotein.

Apart from absorbing red and far-red light, the phytochrome also absorbs blue light. The P_R form of phytochrome is blue while $\mathbf{P_{FR}}$ from is olive- green in colour. But owing to very low concentration, this pigment is not visible in plant tissues. Phytochrome accounts for less than 0.2% of the total extractable protein in etiolated seedlings. None of the two components of phytochrome, i.e., apoprotein and chromophore, can absorb light alone. Phytochromes have been detected in wide range of plants in angiosperms, gymnosperms, byrophytes and algae. Dark grown etiolated seedlings are richest sources of phytochrome where this pigment is especially concentrated in apical meristems.

Phytochromes have directly been detected in different part of seedlings, in roots, cotyledons, hypocotyle, epicotyls, coleoptiles, stems, petioles, leaf blades, vegetative buds, floral

receptacles, inflorescences, developing fruits and seeds. Presence of phytochrome has also been shown indirectly in other plant materials.

Within the cells, phytochrome exists in nucleus and throughout the cytosol. The **chromophore** of phytochrome is synthesized in **plastids** while **apoprotein** is synthesized on **nuclear genome**. Assembly of these two components of phytochrome is autocatalytic and occurs in **cytosol.**

Gibberllins and the flowering response

It is now well known that the gibberellins can induce flowering in long-day plants even under non-inductive short days. It is also definite that the gibberellins alone do not constitute the 'florigen', but it is usually held that the gibberellins are in some way connected with the overall process of flowering.

Importance of photoperiodism

The knowledge of the phenomenon of photoperiodism has been of great practical importance in hybridization experiments. Although the floral hormone 'florigen' has not yet been isolated, the isolation and characterization of this hormone will be utmost economic importance. The phenomenon of photoperiodism is an excellent example of physiological preconditioning (or after-effect) where an external factor *(i.e.*, the photoperiodic stimulus) induces some physiological changes in the plant the effect of which is not immediately visible. It lingers on in the plant and prepares the latter for a certain process (*i.e.*, flowering) which takes place at a considerably later stage during the life history of the plant.

Some Phytochrome mediated photoresponses in Plants

1. Photoperiodism.
2. Seed germination.
3. Elongation of leaf, petiole, stem
4. Sex expression.
5. Bud dormancy
6. Rhizome formation

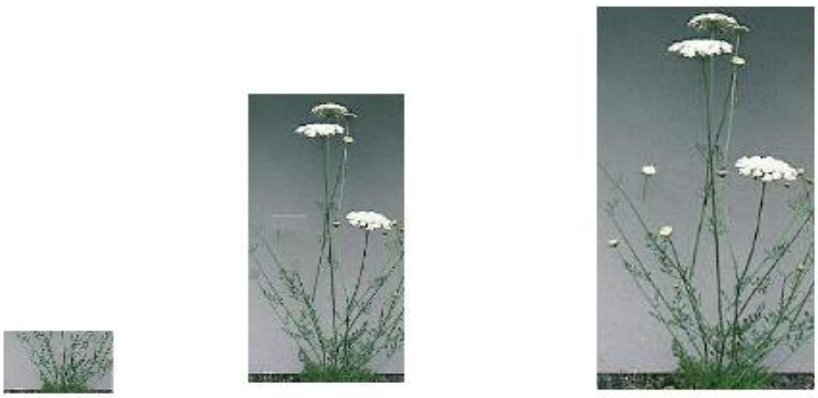
7. Bulb formation
8. Leaf abscission.
9. Epinasty
10. Formation of leaf primordia
11. Flower induction
12. Formation of tracheary elements.
13. Differentiation of stomata.
14. Synthesis of anthocyanins.
15. Increase in protein systhesis.
16. Increase in RNA synthesis.
17. Changes in the rate of fat degradation.
18. Changes in the rate of degradation of reserve proteins
19. Auxin catabolism.
20. Permeability of cell membranes.

Lecture No.11

Vernalization: perception of cold stimulus, presence of floral hormone-mechanisms of vernalization-devernalization-practical utility

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). Due to this, vegetative period is cut short resulting in early flowering.

The effect of cold stimulus on plant is not immediately visible. It is expressed only at a certain later stage in the form of flowering. Thus like the photoperiodism, the phenomenon of vernalization is an excellent example of the physiological preconditioning.



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.

http://www.tutorvista.com/content/biology/biology-iv/plant-growth-movements/flowering-hormone.php

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 vernalization

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.

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.

Oxygen

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

Water

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

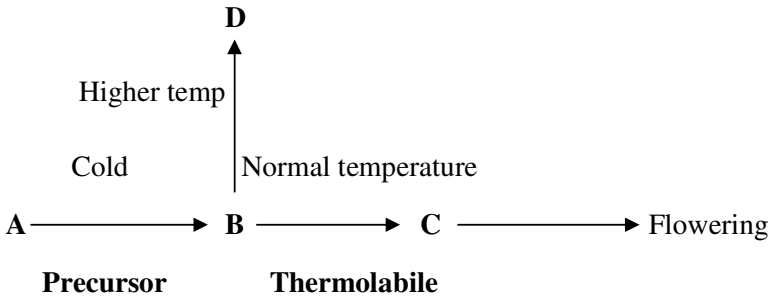
Mechanism of vernalization

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.

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.

Vernalization and gibberellins

The gibberellins are known to replace the low temperature requirement in certain biennial plants such as henbane, where the plant normally remains vegetative and retains its rosette habit during the first growing season and after passing through the winter period flowers in the next season. The gibberellins cause such plants to flower even during the first year**.**

Practical utility of vernalization

- Vernalization shortens the vegetative period of plants
- Vernalization increases the cold resistance of the plants

In colder countries like Russia, wherein the winters are severe, vernalization has been of great importance in agriculture. By this process certain crop plants could be made to escape the harmful effects of severe winters, thus improving the crop production. In warmer countries like India vernalization practice has not been in use mainly because it's a costly process and winters are comparatively not very serve as to harm the crop plants.

Lecture No.12

Physiological basis of training and pruning

Physiological basis of pruning

Pruning is a tool to regulate size and shape of plants to achieve desired architecture of canopy and also reduce foliage density by removal of unproductive branches.

Commonly, trees are pruned annually in two ways. A few shoots or branches that are considered undesirable are removed entirely without leaving any stub. This operation is known as 'thinning out'. The other method which involves removal of terminal portion of the shoots, branches or limb, leaving its basal portion intact, is called 'heading back'. Thinning out involving large limbs as in old and diseased trees is called 'bulk pruning'.

These operations are carried out to divert a part of the plant energy from one part to another. As trees grow older, they should receive relatively more of thinning out and less of heading back. Heading back tends to make trees more compact than thinning out. If a few of the several branches growing close together on the same parent limb are entirely removed or thinned out, the rest of the branches would grow more vigorously. Thinning out results in lesser new shoot growth but more new spurs and fruit bud formation than corresponding severe heading back. Pruning is done with the following specific objectives.

i) To remove surplus branches
ii) To open the trees – maximum sun light interception, so that the fruits will colour more satisfactorily
iii) To train it to some desired form
iv) To remove the dead and diseased limbs,
v) To remove the water sprouts and
vi) To improve fruiting wood and to regulate production of floral buds.
vii) source sink relation

Season of pruning

Little differences are likely to result from pruning at different times during the dormant seasons in deciduous fruit trees though in certain cases, earlier pruning causes earlier foliation in the spring. Late pruning during dormant periods is generally not advocated as it leads to more bleeding than earlier pruning. The exposed cut surface in certain cases may provide an excellent opportunity for infection by some pathogens. For this reason, winter pruning is usually preferred to spring pruning as bleeding will be excess in later period. Summer pruning may have a dwarfing effect or an invigorating influence. A light summer pruning may aid in colouration of fruit in certain species.

The amount of pruning or severity of pruning which is desirable for mature trees differs in different species. The minimum amount, which is common to all, is the removal of broken or diseased branches and those which cross against each other. Diseased branches should be completely removed from the base of the trunk. In other cases annual pruning may be very light in the beginning but after some years it may become necessary to prune heavily. Otherwise, the trees may lack vegetative vigour and make very little growth. Under South Indian conditions, old non-bearing mango trees are pruned to expose the centre portion to sunlight and also crowded terminal shoots are thinned to one or two shoots during August-September. The pome fruits such as apple, plum, pears and peaches are pruned every year in December, January; Jasmines are pruned to 45 cm height from the ground level during the last week of November.

Proper pruning enhances the beauty of almost any landscape tree and shrub, while improper pruning can ruin or greatly reduce its landscape potential. In most cases, it is better not to prune than to do it incorrectly. In nature, plants go years with little or no pruning, but man can ruin what nature has created. By using improper pruning methods healthy plants are often weakened or deformed. In nature, every plant eventually is pruned in some manner. It may be a simple matter of low branches being shaded by higher ones resulting in the formation of a collar around the base of the branch restricting the flow of moisture and nutrients. Eventually the leaves wither and die and the branch then drops off in a high wind or storm. Often, tender new branches of small plants are broken off by wild animals in their quest for food. In the long run, a plant growing naturally assumes the shape that allows it to make the best use of light in a given location and climate. All one needs to do to appreciate a plant's ability to adapt itself to a location is to walk into a wilderness and see the beauty of natural growing plants.

Pruning, like any other skill, requires knowing what you are doing to achieve success. The old idea that anyone with a chain saw or a pruning saw can be a landscape pruner is far from the truth. More trees are killed or ruined each year from improper pruning than by pests. Remember that pruning is the removal or reduction of certain plant parts that are not required, that are no longer effective, or that are of no use to the plant. It is done to supply additional energy for the development of flowers, fruits, and limbs that remain on the plant. Pruning, which has several definitions, essentially involves removing plant parts to improve the health, landscape effect, or value of the plant. Once the objectives are determined and a few basic principles understood, pruning primarily is a matter of common sense.

The necessity for pruning can be reduced or eliminated by selecting the proper plant for the location. Plants that might grow too large for the site, are not entirely hardy, or become unsightly with age should be used wisely and kept to a minimum in the landscape plan. Advances in plant breeding and selection in the nursery industry provide a wide assortment of plants requiring little or no pruning. However, even the most suitable landscape plants often require some pruning.

Reasons for Pruning

- To train the plant
- To maintain plant health
- To improve the quality of flowers, fruit, foliage or stems
- To restrict growth

Plan Approach to Pruning

- Pruning should follow a definite plan. Consider the reason or purpose before cutting begins.
- By making the pruning cuts in a certain order, the total number of cuts is reduced greatly. The skilled pruner first removes all dead, broken, diseased or problem limbs by cutting them at the point of origin or back to a strong lateral branch or shoot. Often, removing this material opens the canopy sufficiently so that no further pruning is necessary.
- The next step in pruning is to make any training cuts needed. By cutting back lateral branches, the tree or shrub is trained to develop a desired shape, to fill in an open area caused by storm or wind damage or to keep it in bounds to fit a given area. To properly train a plant, one should understand its natural growth habit. Always avoid destroying the

natural shape or growth habit when pruning unless maintaining a close watch over the plant, for after a period of time it attempts to assume the more natural growth habit.

- Make additional corrective pruning to eliminate weak or narrow crotches and remove the less desirable central leader where double leaders occur. After these cuts have been made, stand back and take a look at your work. Are there any other corrective pruning cuts necessary? If the amount of wood removed is considerable, further pruning may need to be delayed a year or so. Remove water sprouts unless needed to fill a hole or to shade a large limb until other branches develop.

Physiology of training and pruning

Woody plants are pruned to maintain a desired size and shape and to promote a certain type of growth. Ornamental plants are pruned to improve the aesthetic quality of the plant, but fruit trees are pruned to improve fruit quality by encouraging an appropriate balance between vegetative (wood) and reproductive (fruiting) growth. Annual pruning of fruit trees always reduces yield, but enhances fruit quality. Pruning increases fruit size because excess flower buds are removed and pruning encourages the growth of new shoots with high-quality flower buds. Pruning improves light penetration into the canopy, and light is required for flower-bud development, fruit set and growth, and red color development. Pruning also makes the canopy more open and improves pest control by allowing better spray penetration into the tree; air movement throughout the canopy is increased, which improves drying conditions and reduces severity of many diseases.

This publication describes why plants respond to pruning and other forms of plant manipulation used to train trees. This information applies to all plants, but application to fruit trees is emphasized.

Pruning fruit trees is somewhat of an art based on an understanding of plant physiology and development. In other words, if we understand how plants grow and how they will respond to different types of plant manipulations, we can alter vegetative growth and fruiting to obtain trees and fruit with desirable characteristics.

A basic understanding of certain aspects of plant physiology is a prerequisite to understanding pruning. Unlike animals, plants continue to increase in size throughout their lives. There are only two ways plants can grow.

Primary growth is the increase in length of shoots and roots, and is responsible for increases in canopy height and width.

Secondary growth is the increase in thickness of stems and roots.

Both types of growth require cell division followed by cell enlargement and differentiation.

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Plant Growth

Meristems are regions of cell division and there are two types of plant meristems. An apical meristem is located at the tip of every shoot and root (Figure 1). As cells divide in these apical meristems, the shoots and roots elongate as cells are piled one on another. Behind the region of cell division is a region of cell differentiation, where cells enlarge and differentiate into various tissues. In the axil of each leaf is a small apical meristem called an axillary meristem that forms an axillary bud, which usually remains dormant until well after the subtending leaf is fully developed. An axillary bud may remain dormant or develop into a lateral branch or a flower.

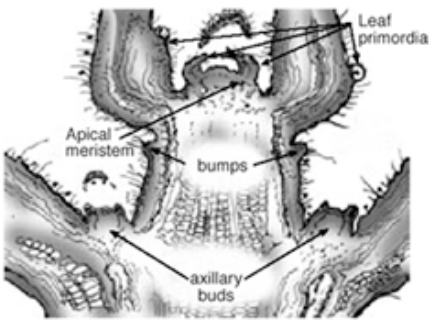

Figure 1. Longitudinal section of shoot tip shows an apical meristem, successively older leaf primordia and axillary bud primordia.

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There are two distinct layers of meristematic tissue within the stem or root responsible for secondary growth, the vascular cambium and the cork cambium (Figure 2). The vascular cambium is a cylinder of specialized cells, usually five to ten cells thick, running the length of the plant, including the roots, and is responsible for the radial growth of plant parts. Phloem cells are produced to the outside of the cambium and xylem cells are produced to the inside of the cambium. Downward transport of sugars, nutrients, and hormones from the top of the tree to the roots occurs in the phloem tissue. Xylem cells are tube shaped, become hollow and die to form a pipe-like system through which water, hormones and mineral nutrients move from the roots to the top of the tree. Most of the radial growth of woody plants is due to activity of the vascular cambium, but a small amount results from activity in another lateral meristem, the cork cambium, located outside the vascular cambium. The cork cambium (phellogen) together with the cork cells, constitute the periderm: a protective layer of suberized dead cork cells forming the bark. Suberization is the impregnation of cell walls of cork tissue with a fatty substance called suberin. Each season new layers of cells are produced and appear as growth rings when viewed in cross-section. Over time, the xylem cells at the center of the trunk or limb are crushed and become nonfunctional as transport pipes, but they do provide structural support to hold the plant upright. While grafting it is important to line up the cambiums of the scion and the rootstock to ensure a successful graft union.

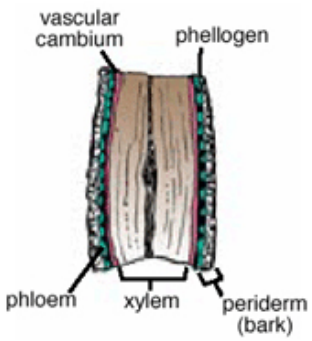

Figure 2. Longitudinal cross-section of a tree trunk shows the vascular and cork cambiums.

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Buds

Buds are important to the vegetative and reproductive growth of trees. Fruit tree training

and, to a lesser extent, pruning primarily involves bud manipulation. Buds are actually undeveloped shoots. When a vegetative bud is sliced longitudinally during the winter and viewed under magnification, the apical meristem at the tip, leaf primordial (developing leaves), axillary meristems, developing axillary buds, and procambial tissue (tissue that will develop into the cambium) are all visible.

Buds on fruit trees usually have about seven leaves and initial shoot elongation in the spring results from cell expansion. During late June and July some of the shoot apices will flatten out and develop into flower buds. Flower buds are actually modified shoots and the various flower tissues (petals, stigmas, anthers, etc.) are actually modified leaves. Although the process of switching from vegetative to reproductive buds is not fully understood, hormones that can be influenced by environmental factors, stresses, and plant nutrition control the process.

There are several things we can do to influence whether or not a bud becomes a flower bud or remains vegetative. In general, factors that favor rapid growth, such as high nitrogen levels in the shoot tissues, inhibit the development of flower buds. Applying growth-promoting plant growth regulators such as gibberellins usually inhibits flower-bud induction, whereas ethylene may promote flower-bud development. Mild stresses such as shoot bending and water stress may also promote flower-bud development.

Producing annual crops of high-quality fruit requires a balance between reproductive and vegetative growth. Fruit producers use various techniques, including pruning, branch bending, and plant growth-regulator sprays, to manipulate tree growth and flowering. Often these techniques affect bud dormancy, so knowledge of buds and bud dormancy is essential if we are to understand how pruning influences tree growth. It is also important to be able to identify the different types of buds on a tree, especially to distinguish between flower and vegetative buds.

Buds may be classified based on location, contents, or activity.

Classification by content Several types of buds commonly develop on fruit trees. Vegetative buds only develop into leafy vegetative shoots. Flower buds produce only flowers. Stone fruit trees (peach, nectarine, apricot, plum, and cherry) produce vegetative buds and flower buds. Apple and pear trees produce vegetative and mixed buds. Both leafy shoots and flowers emerge from mixed buds.

i. Classification by location

Terminal buds are located at the tip of a shoot. On stone fruit trees terminal buds are vegetative buds. Terminal buds on apple and pear trees are usually vegetative; however, some varieties such as Rome Beauty produce mixed buds terminally and are referred to as tip bearers or terminal bearers. Most mixed buds on apple and pear trees are formed terminally on short, less than sixinch, shoots that terminate with a rosette of leaves. These short shoots are called spurs. Lateral buds form in the axils of leaves and are often referred to as lateral buds or axillary buds. On stone-fruit trees, lateral buds may be either vegetative or flower. Nodes on one-year-old shoots may have one to three buds, some of which may be flower buds and others vegetative buds. Flower buds are larger with tips that are relatively round, whereas vegetative buds are small, narrow, and pointed. In the case of apple and pear trees, lateral buds on the previous season's growth are usually vegetative. However, lateral buds on some varieties, especially on the dwarfing rootstocks, may be mixed buds.

ii. Classification by arrangement on the stem

The bud arrangement influences the arrangement of a fruit tree's branches and thus the tree's shape and how easy it is to manage. A node is the joint on a stem where a leaf is or was attached (Figure 3). Axillary buds are located in the axis above where a leaf is attached to the stem. In apples there is usually only one leaf per node, whereas three leaves often arise from a node on peach shoots. When a leaf falls in the autumn, a leaf scar remains just below the axillary bud (Figure 3). Buds are opposite when there are two at the same node but on opposite sides of the stem. Forsythia is an example of a plant with opposite buds. Buds are alternate when there is only one from each node and no one bud is on the same side of the stem as the one next above or below it. Deciduous fruit trees have buds that spiral along a shoot (Figure 4). The spiraling three-dimensional arrangement of leaves around a stem is known as Phyllotaxy and is expressed as a fraction, where the numerator is the number of turns to get to a leaf directly above another and the denominator is the number of buds passed.

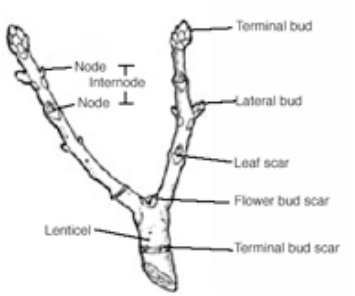

Figure 3. Section of a limb shows nodes, leaf scars, and different types of buds.

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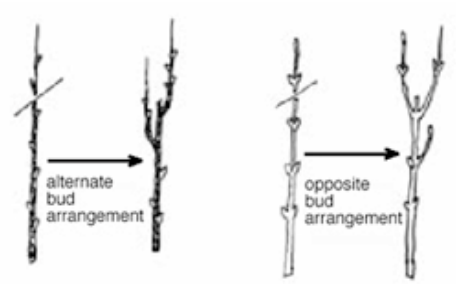

Figure 4. Shoots with alternate arrangement (left) and opposite arrangement (right).

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In each case the shoot has been headed and the diagram to the right of the arrow indicates how the buds respond to the heading cut.

iii. Classification by activity

Buds are dormant when they are not visibly growing. When shoots develop around large pruning cuts, they usually are sprouting from dormant buds (Figure 5). Adventitious buds form irregularly on older portions of a plant and not at the stem tips or in the leaf axils. They form on parts of the root or stem that have no connection to the apical meristems. They may originate from either deep or peripheral tissues. For example, shoots often arise from adventitious buds

growing from callus tissue around wounds. Root suckers (vigorous upright shoots developing from the roots) develop from adventitious buds on the roots.

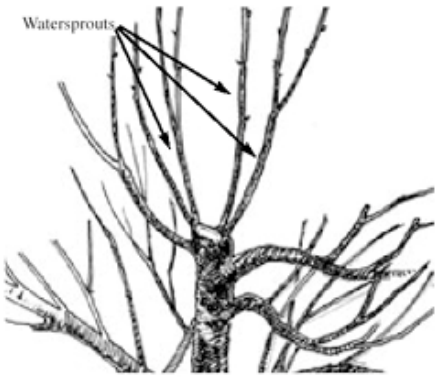

Figure5. Watersprouts developing from adventitious buds around a pruning cut used to lower a tree.

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Plant Hormones

Hormones are substances produced in very small amounts in one part of the plant and transported to another part where they cause a response. Plants produce a number of hormones that control various aspects of growth, such as stem elongation; dormancy of buds and seeds; flowering; fruit set, growth, and ripening; and the response to light and gravity. While pruning, it is useful to consider the activity of the general types of hormones, promoters (gibberellins and cytokinins) and inhibitors (auxins and abscisic acid). Promoters generally cause bud growth, cell division and elongation, and stem growth. Inhibitors are usually associated with dormancy and inhibit shoot development from seeds and buds and may be involved in flower-bud induction. It is often the ratio of promoters and inhibitors, rather than their absolute concentrations, that determines how a plant will grow. The production of plant hormones is usually controlled by environmental conditions such as temperature or day length. Vegetative growth is usually associated with low ratios of inhibitors to promoters and dormancy is usually associated with high ratios of inhibitors to promoters.

Dormancy is a condition characterized by temporary growth cessation and suppressed metabolism. During the winter trees appear not to be growing, but the tissues are alive, there is

metabolic activity, and cells are slowly expanding and differentiating. By early October all the flower parts (petals, stigmas, anthers, etc.) can be seen in a flower bud and vegetative buds contain leaves. During the winter these various tissues continue to enlarge and differentiate. Given favorable growth conditions, some buds will develop into shoots or flowers, but others may remain dormant. By understanding the factors influencing bud dormancy we often can influence certain aspects of tree growth. Buds of deciduous trees go through several stages of dormancy. Results from dormancy research are confusing because plant physiologists have used different terminology to describe the stages of dormancy. Plant physiologists currently describe dormancy in four stages.

Para-dormancy occurs in the mid to late summer when buds do not grow because inhibitors produced in the leaves and terminal buds inhibit bud growth. Para-dormancy can often be overcome by removing leaves (leaf stripping) along a section of a shoot so the axillary buds develop into shoots. Nurserymen often use this technique to produce trees with lateral branches (feathered trees). Using heading cuts to remove the terminal portion of a shoot will allow several axillary buds just below the cut to develop into shoots. Sometimes an application of growth promoters (gibberellins and/or cytokinins) will induce bud growth.

Sometimes axillary buds do not become dormant and develop into shoots within a few days of being formed. Such shoots are referred to as sylleptic shoots and are fairly common on vigorously growing peach trees, but are rarely produced on apple trees (Figure 6).

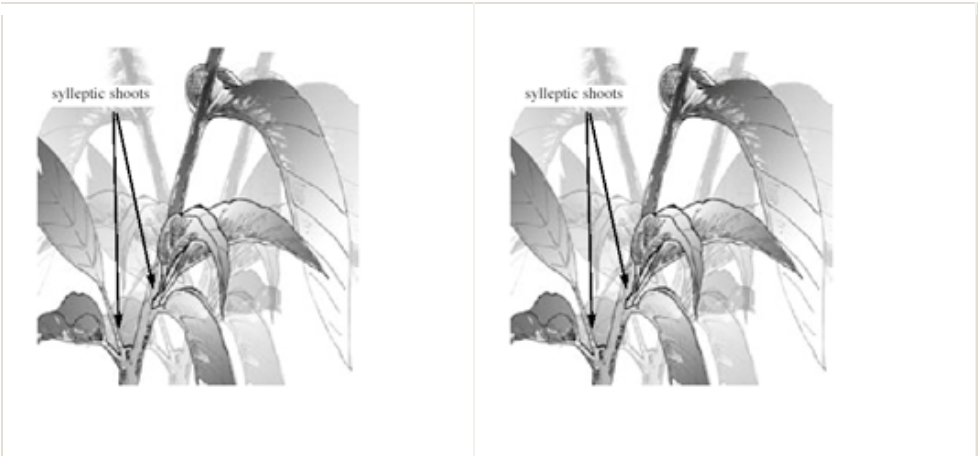

Figure 6. Sylleptic-shoot growth on peaches during the growing season (left) and during the winter (right). Arrows indicate sylleptic shoots.

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Ectodormancy occurs in the early fall, before defoliation, when plants do not grow because the environmental conditions are not conducive for growth. Growth will resume if the plants are exposed to suitable temperatures and day lengths.

Endodormancy occurs during the winter because there are high levels of inhibitors (abscisic acid) within the buds. During this phase of dormancy the trees will not grow even under ideal growing conditions. The concentration of inhibitors declines as buds are exposed to chilling temperatures. Temperatures near 45°F are ideal for chilling, but temperatures between 35° and 55° F will provide some chilling. The chilling requirement to satisfy dormancy for most varieties of apples and peaches grown in Virginia is about 1,000 and 800 hours, respectively. When the chilling requirement is satisfied, the level of inhibitors within the bud is low enough that growth may commence when environmental conditions are appropriate for growth. Avoid planting varieties with chilling requirements less than 800 hours because such varieties usually bloom early and are susceptible to frost.

Eco-dormancy occurs in the late winter, usually by mid January, after the chilling requirement has been satisfied. At this time the trees do not grow because conditions are unsuitable for growth. Growth will commence when trees are exposed to warm temperatures.

Apical dominance is a type of para-dormancy, where axillary bud growth is inhibited in the apical meristematic zone. Axillary buds on fruit trees typically remain dormant for a prolonged period while the main shoot continues to grow. Apical dominance has been studied for more than 80 years, and the exact mechanism is not yet fully understood, but it seems to be controlled by the relative concentrations of inhibitors and promoters. Growth of axillary buds is inhibited by high concentrations of auxin produced by the terminal bud. Auxin moves down the shoot, from cell to cell by gravity, so concentrations are highest near the shoot tip. Promoters are produced in the roots and are transported upward in the tree. Growth of axillary buds may occur at the base of shoots where concentrations of inhibitors are relatively low and concentrations of promoters are relatively high.

You can overcome apical dominance by removing the shoot tip, which is the source of auxin (Figure 7). The three or four buds immediately below a heading cut usually develop into shoots. Pinching annual plants to induce branching is a form of heading. Another way to overcome apical dominance is to notch buds. Notching involves cutting through the bark to hard wood, with a knife or hacksaw blade at about bloom time, just above a bud. The cut interrupts the downward flow of inhibitors, but not the upward flow of promoters, and releases the bud from dormancy. On vigorous upright one-year-old shoots, notching often successfully overcomes dormancy in about 70 percent of the buds. Sometimes apical dominance can be overcome by spraying shoots with promoters (gibberellins and/or cytokinins) just before bloom time.

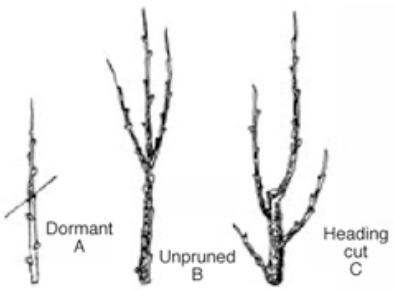

Figure 7. One way to overcome apical dominance and inducing branching where we want branching is to head the shoot (A). If the shoot is not headed, the top several buds will develop into shoots (B). If the shoot is headed, several buds below the heading cut will develop into shoots (C).

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Shoot bending

Shoots bend in response to an auxin gradient within the shoot. Everyone who has grown plants in the house has noticed that plants tend to grow towards the light. This phenomenon is known as photomorphism and is caused by varying concentrations of auxin in different sides of a stem or shoot. Auxin causes cells to elongate, but auxin is destroyed by light. Therefore, there is a higher concentration of auxin on the dark side of a shoot and the cells on the dark side elongate more than cells on the sunny side of the shoot, causing the shoot to bend towards the light (Figure 8).

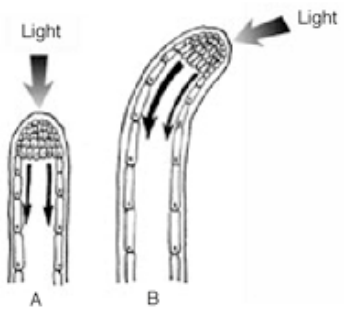

Figure 8. Photomorphism is the bending of a shoot towards the light.

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The auxin concentration is highest on the dark side of the stem and causes cells on that side to elongate, resulting in stem curvature.

Tree fruit producers have noticed a similar phenomenon where the tips of growing branches tend to bend upward, even when the branch was physically oriented to the horizontal. This condition, known as gravimorphism, is also caused by an auxin gradient within the branch

in response to gravity. Auxin flows by gravity to the lower side of a limb. The subsequent accumulation of auxin is responsible for increased cell elongation on the underside of the limb, and the growing tip bends upward.

Another consequence of gravimorphism is the development of watersprouts from the upper surface of horizontally oriented limbs. Watersprouts are vertically growing shoots that develop from the upper surfaces of branches or near pruning cuts. High auxin concentrations on the underside of the limb inhibits growth of buds on the underside of the limb, but the concentration of auxin on the upper side of the limb is inadequate to inhibit bud growth and many of these buds develop into watersprouts. Watersprouts are usually undesirable and their development can be suppressed by orienting limbs no more than 45 degrees from the vertical. Fruit trees are sometimes trained as espalier (tree fence). There are several ways to espalier trees, but one method involves orienting limbs to a horizontal position. This system induces many watersprouts along the length of the branches. Watersprout development can be greatly suppressed by orienting limbs 45 to 60 degrees above horizontal (Figure 9).

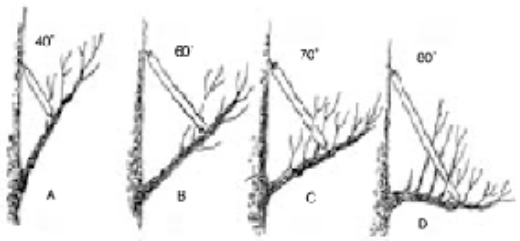

Figure 9. Auxin distribution within a stem is controlled by gravity. When limbs are oriented from vertical to about 60 degrees from vertical, auxin is distributed fairly evenly around the limb and buds develop into shoots fairly symmetrically around the limb (A and B).

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Auxin accumulates on the underside of flat limbs (C and D) and inhibits growth of buds on the underside. Auxin concentration is low on the upper side and buds are not inhibited and develop into strong watersprouts.

Reducing tree height by cutting into large diameter branches or trunks often results in the development of vigorous watersprouts around the cut. There are buds buried in the bark that normally remain dormant. However, a severe pruning cut will release these buds from dormancy.

Additional Pruning Facts

Pruning is a dwarfing process pruning increases vegetative growth near the pruning cut and this gives the illusion that pruning stimulates growth. However, the weight of a tree that was pruned annually is always less than the weight of a nonpruned tree.

Pruning reduces yield

Pruning removes wood with flower buds, and thus potential fruit. Yield from pruned trees is nearly always less than yield from nonpruned trees, but fruit quality is improved by pruning. Pruning improves fruit size by increasing the amount of leaf area per fruit. Pruning improves light distribution throughout the tree, which is important for the development of fruit red color and sugar levels.

Pruning delays fruiting

Pruning encourages vegetative growth rather than reproductive growth in young trees. A nonpruned tree will always flower and produce fruit earlier in the life of the tree than a pruned tree. The reason young trees are pruned is to induce branches to develop where they are wanted and to develop a strong tree structure that will support large crops as the tree matures. As a tree matures the physiology changes from vegetative growth to reproductive growth. To obtain high annual yields of mature trees, it is important to minimize fruiting until trees have nearly filled their space. Pruning is one technique used to delay fruiting of young trees.

Summer pruning

Summer pruning involves the selective removal of leafy shoots during the growing season. Responses to summer pruning vary with time of pruning, severity of pruning, tree vigor, geographical location, and variety. Several researchers evaluated summer pruning during the 1980s and several general statements can be made about the practice.

Summer pruning reduces within-tree shade and usually improves fruit red color development and sometimes improves flower bud development. Summer pruning removes leaves that produce photosynthates (sugars) for growth of all tree parts. Summer pruning sometimes reduces fruit size and sugar levels.

Due to reduced whole-tree photosynthesis, summer pruning suppresses late-season trunk enlargement and root growth.

Summer pruning does not suppress shoot elongation the following season. Summer pruning reduces late-season photosynthesis, and theoretically should reduce the accumulation of reserve carbohydrates within the tree that are used for early season growth. However, results from most pruning experiments indicate that the response to a certain type of pruning cut will be the same regardless of the time of year the cut was made.

Summary

Pruning is an important orchard practice because pruning can influence fruit quality and the balance between vegetative growth and fruiting. Successful pruners observe how plants respond to various types of plant manipulation, including pruning. Profitable fruit production requires an understanding of plant physiology, and how pruning alters the physiology of the plant. For further information concerning how to prune fruit trees, see the Extension publications that provide information on how to prune and train apple and peach trees.

Canopy Regulation in Fruit Crops

Canopy management

Canopy in a fruit tree refers to its physical composition comprising of stem, branches, shoots and leaves. The canopy density is determined by the number and size of the leaves, architecture of stem, branches and shoots. Canopy management of the fruit tree deals with the development and maintenance of their structure in relation to the size and shape for the maximum productivity and quality. The basic concept in canopy management of a perennial tree is to make the best use of the land, the climatic factors for an increased productivity in a three

dimensional approach. Tree vigour, light, temperature and humidity play a vital role in the production and quality of the fruits.

The major objective is to achieve maximum productivity in a shortest period with out adversely affecting tree health and bearing of the orchard. The natural tree canopy of the fruit tree varies greatly from species to species and cultivar to cultivar. The size, shape and volume of canopy are affected by climate, planting density, rootstock, method of propagation, training, pruning, regularity of bearing, soil type, nutrition, irrigation, intercrop, growth regulators used, diseases, pests, environmental pollution etc.,

The crux of the canopy management lies in the fact, as to how best we manipulate the tree vigour and use the available sunlight and temperature to increase the productivity and quantity and minimize the adverse effects of weather parameters. Some of the basic principles in canopy management are as follows.

1) Maximum utilization of the light
2) Avoidance of the build up of micro-climate congenial for the diseases and pests
3) Convenience in varying out the cultural operations, maximizing the productivity and quality
4) Economy in obtaining the required canopy architecture.

Light is an important factor in production of fruit. It has a role in flower induction as well as in fruit development through carbohydrate synthesis. While increased assimilates in the shoots is a pre-requisite for flowering in mango and other fruits generally, high yield of quality fruits are attributed to high light interception and distribution in the tree canopy. The fruit yield is related to light interception, whereas fruit quality is a function of light distribution. Light interception is influenced by plant density, canopy shape, canopy leaf area index and can be raised by increasing the density of foliage in the canopy, the height of the tree and number of tress per hectare. Light intensity decreases, within the tree canopy as the outer portion shades the inner canopy. Light exposure influences flower bud differentiation, fruit set, fruit colour and quality. In the canopy management, major emphasis is usually required to reduce the excessive canopy shading and increase the air circulation in the fruiting region.

The practices used to accomplish these objectives are:

a) Control of tree vigour

b) Reduction of canopy shading
c) Training and pruning system to increase light interception and distribution.

Light was found to perform a triggering action in the process of fruit bud differentiation in grapes. Failure of the flowering in mango trees with dense canopies (Buronkar and Gunjate, 1991) and opening of the canopies through pruning (Madhavarao and Shanmugavelu, 1976), (Rameswar, 1989) support indirectly the role of light in fruits bud formation in mango. However, the light dependence for the flower bud formation is not the same in different varieties. While, White Riesling variety of the grape requires less light intensity, Thompson Seedless requires less light for the fruit bud formation. Hogher light intensities of more than 3,600 ft candles and temperature above 35^{o}C are favourable for the bud fruitfulness in Thompson Seedless grape.

The light utilized by the plants for the photosynthesis corresponds to 400 to 700 mm of the electro-magnetic radiation from the sun. Kriedmann and Smart 1971 reported that the photosynthesis in grapes rapidly increases upto the light intensity of 5,000 ft (200 watts/m^2). The light compensation point, at which the rate of photosynthesis, is just the equal to the rate of respiration in Thompson Seedless grape is 125 ft candles (5 watts/m^2). Leaves at the light regimes of lower than the compensation point are the liabilities to the plant. A leaf absorbs more than 90 per cent of the solar radiation depending upon its thickness. Even if the full sunlight in a given locality is 12,000 ft candles, the third layer of leaves in a tree canopy would receive the light at a lesser intensity than the compensation point. Therefore, the tree canopy architecture has to be so managed that every leaf gets light at the intensities, which are more than the compensation point.

Close planting of the trees and the development of dense canopies may alter the micro-climate around the tree canopy. Temperature and light regimes decrease, while humidity increases. The incidence of powdery mildew will be more under the low temperature and in shaded conditions. *Bortrytis* rot of the bunches was observed to be less in the vines with exposed canopy. Low temperature and the high humidity caused by dense canopies in grape was found to favour the incidence and spread of the downy mildew. The efficacy of plant protection measures will be reduced, when the canopy is dense and the trees are tall. Canopy size and shape should be such, so that the cultural operations could be carried out in an orchard with ease and mechanization of some operations is possible. They primary aim of the canopy management is to

increase the productivity per unit area, quality of the fruit and to reduce the cost of production. The canopy architecture should be easy and less expensive.

Ideal canopy architecture

Ideal canopy architecture shoud fulfill as many as possible principles involved in canopy management. *i.e.*, the canopy size should be dwarf, spreading and open in mango and guava. In order to obtain more yields per unit area of the land, it is desirable to have the required surface area per canopy volume by increasing the canopy height. But due to inconvenience in carrying out the cultural operations including harvest, the canopy height should be at manageable level.

Based on the correlation of tree morphological characters with the fruit yield and quantity, dwarf and spreading trees with larger trunks are the ideotypes in guava (Shikhamany *et al.,* 1977). The standards for an ideal canopy for grapes cv. Thompson Seedless are as follows (Shikhamany, 1983):

Stem height - 135cm Diameter - 7.5	Cane number - 5 per m^2 Cane thickness-8 to 10 mm
Cordon length - 90cm	Leaf number per bearing shoot - 12 to 15
Shoot orientation during the growth season- 35 to 45 with the ground surfaces	Shooting orientation during the fruiting season- 35-40^oC with the ground surface upto a length of 90cm and parallel to the ground surface beyoung 90 cm

Tools for the canopy management

Canopy architecture is a natural expression of the genetic make up of a tree. Genotypes vary in the canopy size and shape. However, the size and shape of the canopy may also be manipulated through various means. Some details of a few of them are given here under.

Training

Training of the perennial trees to the open vase centre is an age old practice to harvest the advantage of the light and ventilation. Basically, the training is a potential tool to manage the

canopy architecture of a plant with weak stem like grape vine. Bower system of the training has been found to be the best in tropics throughout the world. Although, it is an expensive training system associated with the reduced light and temperature in vine canopy, increased humidity and disease incidence, it is inevitable for exploiting fully the productive potential of the grape cine in tropics, where the phenomenon of the apical dominance is more pronounced. It is possible to develop as many as 10 shoots/m^2 by subdividing the apices growing in horizontal plain. Vertical canopies, which envisage the best utilization of the light and the minimization of building up of a high humidity in vine canopy, do not have provision to increase the number of fruiting units per unit area. The best way to manage the canopy in grape vines is to develop diageotropic canopies and increase the fruitfulness of the buds and consequently the cluster:cane ratio.

Pruning

Pruning is a tool to regulate the tree size and shape to achieve a desired architecture of the canopy and also to reduce the foliage density by removing the unproductive branches of a tree. Shanmugavelu and Selvarajan, 1985 observed an increased fruiting in Himayunddin, Rumani and Kalepad varieties of mango. Pruning in mango was favourable for flowering by redistribution of endogenous harmones (Madhavarao and Shanmugavelu, 1975) and increasing the total phenolic contents in shoots (Chacko, 1968). The incidence of mango malformation was also reduced by reducing the foliage density by pruning. In mango annual topping or hedging or the combination of both, effectively controlled tree size but reduced the yields. On contrasts, topping plus biannual hedging although controlling vegetative growth to a lesser degree than annual pruning, produced yield similar to those of control trees. Topping at 15^o or 30^o produced better results than topping at 0^o (Table 1). Hedging one side per year or all four sides every two years resulted in higher yields than hedging two sides per year (Table 2) (Victor and Nunez, 1997).

Table 1. Effect of topping angle on growth and yield of mature "Tommy Atkins" mango trees

Topping Angle	Growth				Yield (kg/tree)			Cumulative
	Height (m)		Canopy Vol(m^3)					
	1994	1995	1994	1995	1993	1994	1995	
0^o	6.22 b	6.30 b	147 c	122 b	139 b	240 ab	233 b	612
15^o	6.83 b	7.10 b	299 b	158 b	187 b	283 a	248 b	718
30^o	6.78 b	6.70 b	210 b	136 b	141 b	250 ab	242 b	633
Control	7.80 a	8.40 a	355 a	317 a	214 a	224 a	347 b	785

Means separation within columns by Tukey's multiple range test, 5% level.

Table 2. Hedging intensity effect on growth and yield of mature "Tommy Atkins" mango trees

Topping Angle	Growth				Yield (kg/tree)			Cumulative
	Height (m)		Canopy Vol(m^3)					
	1994	1995	1994	1995	1993	1994	1995	
One side (AN)	6.57 b	5.9 b	109 c	104 b	69 b	172	217 b	458
Two side (AN)	6.43 b	6.10 b	103 c	113 b	128 b	140	265 b	533
Four sides (AN)	6.57 b	6.10 b	215 b	136 b	85 b	235	245 b	565
Control	7.80 a	8.4 a	355 a	317 a	213 a	224	347 a	784

(X): An = Annual;

Means separation within columns by Tukey's multiple range test, 5% level.

In Africa for mango size maintenance, pruning is performed shortly after harvest. The aim is to remove the growth which occurred after the previous harvest by heading all of the braches back. Size maintenance pruning is performed by hand or by mechanically hedging and may only be required every second or third year in cultivars or situations where yearly canopy expansion is not substantial.

Yield in citrus could also be increased by removing the upright branches and encouraging the horizontal ones by pruning (Goswami *et al.,* 1993). While, pruning of one-year-old shoots to their half length has been recommended to increase the yields in mandarin, skirt pruning at a length of 1 m could also increase the yield in Washington Navel sweet orange. Pruning and shoot topping are the regular practices to shape the canopy and to promote fruiting and ripening in grape.

Lecture No.13

Source and Sink Relationship

Plant needs an effective transport system for translocation of their photoassimilates from the area of synthesis (source) to area of utilization (sink). Every plants needs an effective transport system for the translocation of their photoassimilates from the areas of synthesis(source) to areas of utilization(sink). For instance during germination, stored assimilates in the seed is mobilized and moved to active meristems for leaf, stem and root development and soon the seedlings become autotrophic. Assimilates produced by green tissue is translocated throughout the plant for growth, development, storage and maintenance. The division of assimilates among these process termed partitioning determines the crop production. The partitioning of assimilates and inorganic nutrients can affect both the efficiency of phloem transport, dry matter production and the partition of matter in the harvest.

In plant growth & development, materials are moved from source to sink, primarily through xylem & phloem.

- Assimilates produced by green tissues is translocated throughout plant for growth, development, storage and cell maintenance .The division of assimilates among these process is termed as partitioning
- It determine the dry matter production and productivity of crop

How do you increase source and sink activity

Source is increased by

- Recommended dose of fertilizer
- Application of growth regulating substance
- Proper irrigation
- Removal of diseased leaves
- Proper plant protective measures

Sink activity is increased by

- Pruning
- Thinning
- Clipping
- Removal of excess vegetative growth
- Dwarfing in the case of fruit crops (Applying growth retardants)
- Girdling
- Notching
- Bending
- Application of growth regulating chemicals (booster) at the time flowering, fruit settings, maturation and ripening.
- Application of micro nutrients at the time flowering, fruit settings

Phloem transport

- In plant growth & development materials are moved from source to sink through xylem (acropetal) and phloem (basipetal & acropetal)
- Lateral movement in both xylem &phloem takes place through plasmodesmata
- Phloem –sucrose, amino acids, low quantities of growth regulators nucleotides inorganic & systemic pesticides

The carbohydrates especially sucrose, nitrogenous substances & low quantities of growth regulators , nucleotides, inorganic substances & systemic pesticides move through phloem. But substances like reducing sugars, contact herbicides, proteins, polysaccharides, Ca, iron ,& most micro nutrients do not move in phloem. The mechanism of translocation follows munch's mass flow theory.

Translocation rates

- The rate of movement of compound is controlled by the rate of acceptance by sink
- The rate of movement of substance is measured by labeled isotopes ^{14}C, ^{32}P, ^{40}K, ^{55}Fe, ^{45}Ca.

- Phloem size seems to develop according to the size of the source or sink it serves. Size and volume of phloem limits the flow from source to sink
- Larger the leaf area larger is the cross-sectional phloem area.
- Translocation rate differs among the species of crop plant in C_3 &C_4 plants
- Leaves of C_4 plants have higher CER than C_3 plants
- The improved export of assimilates in C_4 plant may be due to Krantz anatomy
- Bio molecules in the signaling pathways have greater role in the source sink relations.

However there is evidence to indicate that improved export might be related more to higher CO_2 exchange rates than to leaf anatomy.

Phloem loading & unloading can be rate limiting & can affect translocation.

Assimilate partitioning

- Partitioning of assimilate is generally to the sink closest to the source
- Eg: upper leaves export principally to shoot apex. Lower leaves to roots & middle leaves to both

Source & Sink relationship

- The photosynthetic source cells produce the sugars, which can move symplastically to sieve tubes.
- Phloem loading increase sugar concentration to sieve tubes above apoplast
- Sink carbohydrates are utilized
- The build up of sugars in source &removal in sink establish a hydrostatic pressure which moves water &sugars
- Increase of photosynthetic rate-increase in Hydrostatic pressure & Translocation rate
- Sink have the ability to utilize more assimilate.

- If unable to increase in production of assimilate – accumulate and reduce photosynthesis

Hormonal action

- The effect of hormones on sink cell have an effect on partitioning
- IAA, Cytokinin, Ethylene, GA
- They influence –initiation, development, abortion of flowers and seeds.
- Significant effects on source sink relationship in crops.

Assimilate partitioning during vegetative phase

- Leaves, green tissues- original sources
- Some remains in green tissues-cell maintenance
- If slow –converted to starch or some storage materials
- Rest transported to vegetative sinks –roots stem leaves.

The proportion of assimilates partitioned to these 3 organs can influence plant growth & productivity depends upon the nature of crop either root, stem or leaf forms the main economic portion of the crop.

- Young leaves –matured leaves
- Self sufficient export 60-80% -to other parts &to older leaves

During reproductive phase

- Reproductive growth primary part of plant
- Fruits, seeds, flowers, tubers resultant products of larger partitioning of assimilate from vegetative (source) to sink
- In determinant species leaf &stem growth cease at flowering
- Indeterminate species – both vegetative reproductive occur simultaneously

- If much vegetative growth during reproductive phase, yield reduces
- As size of source increase photosynthesis increases

Harvest index

- Two useful terms used to describe partitioning of dry matter by the plant are
- Economic yield is used to refer to the volume or weight of those plant organs that constitute the product of economic value.
- Biological yield proposed by Niche poroviah in 1960 to represent the total dry matter accumulation of a plant's system.
- The proportion of biological yield represented by economic yield – harvest index

$$HI = EY/BY*100$$

Crop yield can be increased either by increasing the total dry matter produced or by increasing the proportion of economic yield or both. More partitioning leads to increase of HI, increase of crop yield.

Yield component

- Grain yield –product of a no of sub fractions called yield component.

$$Y=Nr\ Ng\ Wg$$

- Nr-No of reproductive units
- Ng-No of grains/ reproductive units
- Wg-Avg,wt./grain
- Yield components are affected by management, genotype & environment.

Remobilization

- The movement of compounds from an area where they were once deposited to an area where they can be reutilized – Remobilization

- It occurs in organic & inorganic components
- During leaf senescence carbohydrate, nitrogenous compounds, metabolic nutrients are remobilized.

Grain filling

For the development of grain photosynthate come from 3 sources:

- Current leaf photosynthesis
- From non-leaf parts
- Remobilization deposited in other plant organ.
- During drought grain yield reduces.

Lecture No.14

Regulation of fruit set and development

It is well documented that flower and fruit set have a major impact on fruit quality and regularity of bearing of fruit trees and that flower and fruit thinning help to avoid alternate bearing and improve fruit quality. Efficient, chemical-free flower and fruit thinning methods would help to further reduce chemical inputs in integrated fruit production and to reduce production costs and alternate bearing in fruit orchards

Apple

Fankhauser and Schumacher (1984), Studies on the influence of growth regulators on fruit development, brought out important findings:

- Experiments have shown that spraying with Amid affects trees even with light bloom and therefore such trees should not be thinned.
- Treatment with Alar and PP333 in spring reduces the size of fruit. With the variety 'Gravenstein' we have observed that PP333 has the effect of significantly diminishing the fruit size, but it enhances bitter pit.
- Fruit treated with Alar had the better skin color at harvest time than those treated with PP333, which showed a high percentage of green fruit.
- PP333 produced stronger inhibition of shoot growth than Alar. However this regulator seems to be very persistent in the soil
- Reduced fruit drop in June on AVG treated 'Jonathan' trees.
- The fruit load was much higher but the fruit size was strongly reduced.
- The positive effect was the reduction of internal breakdown.

Costa, G., Baraldi, R. and Bagni, N. 1984. INFLUENCE OF PUTRESCINE ON FRUIT-SET OF APPLE (CV "RUBY SPUR"). Acta Hort. (ISHS) 149:189-196

- Putrescine was sprayed on "Ruby spur/MM 106" apple trees, at different times of application in order to increase fruit set.

- The putrescine concentration of 10^{-3}M applied at 20–30% open flowers or of 10^{-2}M at full bloom induced the best fruit set.
- Also the fruit growth was generally increased by putrescine treatment. As far as the uptake study is concerned the amount of polyamines (putrescine, spermidine, spermine) found in samples of flowers and leaves sprayed in open field with 10^{-3}M solution of each polyamine reached a maximum value within 1 hour after treatment.

Bertschinger, L., Stadler, W., Stadler, P., Weibel, F. and Schumacher, R. 1998. NEW METHODS OF ENVIRONMENTALLY SAFE REGULATION OF FLOWER AND FRUIT SET AND OF ALTERNATE BEARING OF THE APPLE CROP. Acta Hort. (ISHS) 466:65-70

- Several approaches i) Various ways of hand flower thinning, ii) mechanical flower thinning with a thinning machine, iii) shading trees from light admittance to enhance fruit abscission, and iv) pruning at different dates to regulate flower induction and bud differentiation.
- Hand thinning strategies were effective in regulating alternate bearing with cvs. Boskoop and Elstar which tend to crop every second year and are difficult to regulate, but this approach is labour intensive and cultivar particularities need to be respected.
- Some practical recommendations can be made regarding the use of a thinning machine.
- Shading of orchard apple trees at particular dates affected June drop significantely, but further basic studies are required to value the potential of this approach for practical apple growing.

Grochowska, M.J., Karaszewska, A., Jankowska, B. and Mika, A. 1984. THE PATTERN OF HORMONES OF INTACT APPLE SHOOTS AND ITS CHANGES AFTER SPRAYING WITH GROWTH REGULATORS. Acta Hort. (ISHS) 149:25-38

- Intact spurs and annual shoots of apple trees /McIntosh and Jonathan cvs/ and those sprayed with growth regulators /McIntosh and Melba cvs/ were analyzed for endogenous levels of auxin, gibberellin and cytokinin-like substances on six or four dates, beginning from the fourth week after full bloom.

- Hormones extracted with ethanol from soft, growing parts of spurs and annual shoots were purified, and partitioned by TLC then determined by specific bioassays.
- No significant differences were found in auxin levels between fruiting and non-fruiting spurs.
- A high level of gibberellins occurred in the former while cytokinins prevailed in the latter.
- Treatments of the trees with gibberellic acid brought about a decrease in cytokinin level of the spurs.
- A mixture of succinic acid-2, 2-dimethylhydrazide /SADH/ and Ethrel /1000 and 500 mg/l/ caused an abrupt decrease in endogenous gibberellins and an increase in cytokinins, whose level reached a maximum about a month later.
- In annual shoots an increase in cytokinins after treatments with growth retardants was preceeded by their fall.
- A direct relationship was found between the ratio of endogenous cytokinins to gibberellins and the ability of the shoots to produce flower buds.

Grapes

Ben-Arie, R., Sarig, P., Cohen-Ahdut, Y., Zutkhi, Y., Sonego, L., Kapulonov, T. and Lisker, N. 1998. CPPU AND GA_3 EFFECTS ON PRE- AND POST-HARVEST QUALITY OF SEEDLESS AND SEEDED GRAPES. Acta Hort. (ISHS) 463:349-358

- Berry size of seedless grapes is generally increased by application of GA_3 sprays at the time of fruit set.
- For certain cultivars a number of applications is required to obtain a commercially acceptable sized berry, and this may have deleterious effects on fruit bud initiation for the following year.
- It has been found that a similar increase in berry size can be obtained by a single application of CPPU to Perlette, Superior and Thompson Seedless cultivars As with GA_3, fruit ripening and maturation were delayed following CPPU application, sometimes even more severely than with GA_3.
- However, other aspects of development of the cluster were affected differently by each growth regulator.

- Such aspects include the growth of the rachis and pedicels, berry shatter after harvest and susceptibility of the berry to fungal attack and decay
- These differences may be attributed to the anatomical effects of the regulators, in that CPPU increased the number and density of cells, whereas GA_3 enhanced cellular expansion and decreased cell density
- Each growth regulator also increased the size of seeded berries of cultivar Zeiny and fruit ripening and resistance to decay were similarly affected.
- When a combination of both compounds was applied, there were no additive effects on berry size, berry firmness and fruit ripening, but the relative resistance to decay conferred by CPPU, was eliminated by addition of GA_3.
- Anatomical observations showed that the cell area and skin thickness of berries treated with both regulators were the same as for berries treated with GAR alone.

Mango

Singh, Z. and Agrez, V. 2002. FRUIT SET, RETENTION AND YIELD OF MANGO IN RELATION TO ETHYLENE. Acta Hort. (ISHS) 575:805-811

- The role of ethylene in fruit set and retention of 'Kensington Pride' mango was investigated employing exogenous applications of ethylene and its biosynthesis and action inhibitors including aminoethoxyvinylglycine (AVG), and aminooxyacetic acid (AOA) and cobalt sulphate ($CoSO_4$) and ethylene action silver thiosulphate (STS).
- A spray application of ethylene biosynthesis inhibitors was more effective at increasing initial, final fruit set and fruit retention (120, 42 and 1.8 fruits/panicle), when compared with the ethylene action inhibitor STS (104, 38.9 and 1.7 fruits/panicle) respectively, when applied to fully-grown panicles before anthesis.
- Significant direct positive linear correlations were observed between both initial and final fruit set and fruit retention, in all weeks after initial fruit. Exogenous application of ethrel significantly reduced fruit set (up to 76%). Fruit yield was significantly improved with all ethylene biosynthesis and action inhibitors.
- However, the results indicate that increasing initial or final fruit set has no effect on fruit yield, although, there was a significant direct correlation between final fruit retention and fruit yield.

- The increase in fruit yield with treatments of ethylene biosynthesis and action inhibitors may be due to their effects on improving fruit retention.
- No deleterious effects on quality were observed with the single spray application of ethylene biosynthesis and action inhibitors before anthesis.
- The promotion of fruit set with the inhibitors of endogenous ethylene biosynthesis and it's action and reduction in fruit set with the exogenous application of ethrel showed that endogenous ethylene plays an important role in the fruit set of mango.
- In conclusion, single exogenous spray applications of $CoSO_4$ (200mg/L) to fully-grown panicles before anthesis, was most effective for improving fruit set, retention and yield.

Jutamanee, K., Eoomkham, S., Pichakum, A., Krisanapook, K. and Phavaphutanon, L. 2002. EFFECTS OF CALCIUM, BORON AND SORBITOL ON POLLINATION AND FRUIT SET IN MANGO CV. NAMDOKMAI. Acta Hort. (ISHS) 575:829-834

- The effects of calcium, boron and sorbitol on pollen germination in vitro, pollen tube growth on stigma and fruit set in mango cv. Namdokmai were investigated.
- Both commercial and technical grades of calcium nitrate and boric acid were used in this study, while only purified sorbitol was employed.
- All chemicals were sprayed onto 5-cm long mango inflorescences.
- When 50% of the flowers had bloomed, pollen was collected for in vitro observations (Brewbaker and Kwack agar medium).
- Pollen tube growth on the stigma was examined by the aniline blue fluorescence method, every 6-hours for 48 hours from 6 hours after hand pollination.
- Fruit set was examined every 3 days for 45 days after full bloom.
- The results showed that the applied chemicals had no effect on pollen germination or pollen tube growth.
- The percentage of pollen germination in all treatments was 44.52% to 54.16%, and pollen tubes took 24 to 30 hours to reach the stigma ends.
- Although, calcium, boron and sorbitol did not influence pollen germination and tube growth, they did induce more fruit set in this mango cultivar.
- Therefore, it is anticipated that these chemicals may exert other effects on the fruitset.

Singh, Z. and Janes, J. 2000. REGULATION OF FRUIT SET AND RETENTION IN MANGO WITH EXOGENOUS APPLICATION OF POLYAMINES AND THEIR BIOSYNTHESIS INHIBITORS. Acta Hort. (ISHS) 509:675-680

- Aqueous solutions of spermine, spermidine and putrescine of different concentrations (10^{-3}, 10^{-4}, 10^{-5} M) and spermine (10^{-3}, 10^{-4}, 10^{-5} M) were sprayed onto fully grown panicles prior to the anthesis in "Kensington Pride" and cvs Haden, Kent, Glenn and Kensington Pride of mango (*Mangifera indica* L.) respectively to study the effects on fruit set and retention.
- The effects of different concentrations (0, 1, 10, 100, 250 and 500 μM) of DFMO (DL-

  -difluoro-methylornithine) or MGBG (methylglyoxal-bisguanyl hydrazone), inhibitors of biosynthesis of polyamines on initial and final fruit set in "Kensington Pride" mango were also studied.
- A single spray application of spermine onto the fully-grown panicles prior to the anthesis resulted in higher mean fruit set in ‘Kensington Pride’ as compared to putrescine, and spermidine. Spermine (10^{-4}M) was most effective in increasing mean fruit set as compared to control and all other treatments.
- The improvement in mean fruit set with the spermine treatments varied greatly among different mango cultivars.
- The treatment of spermine (10^{-4} M), when applied onto the fully-grown panicles prior to the anthesis increased fruit set in cvs Haden, Kent and Kensington Pride, where as (10^{-5} M) was the most effective in stimulating fruit set in Glenn.
- The increase in fruit retention with spermine was similar among all the cultivars.
- The treatments of inhibitors of biosynthesis of polyamines reduced the initial and final fruit set in "Kensington Pride" mango, when applied onto the fully-grown panicles prior to the anthesis.
- Spray application of spermine (10^{-4} M) onto fully-grown panicles prior to anthesis was most effective in increasing the final fruit retention in all the cultivars.

Pear

Herrero, M. 1984. EFFECT OF TIME OF GA_3 TREATMENT ON 'AGUA DE ARANJUEZ' PEAR FRUIT SET. Acta Hort. (ISHS) 149:211-216

- GA_3, at 10 ppm, was applied at three different stages of flower development.
- All treatments induced parthenocarpic set, although application at balloon and anthesis resulted in a higher set than at petal fall.
- The latter treatment produced fruits similar to seeded fruits, while the two previous applications gave elongated fruits.
- No differences in maturity could be observed between treatments or when comparing with seeded fruits.
- Ovule development at different floral stages has been histologically investigated.
- GA_3 induced fruit set either with mature or immature embryo sacs.
- The differences in % set may be related to the stage of development of the nucellus.

Marcelle, R.D. 1984. EFFECTS OF GA 3, BA AND GROWTH RETARDANTS ON FRUIT SET IN THE PEAR CULTIVAR 'DOYENNE DU COMICE'. Acta Hort. (ISHS) 149:225-229

- In the pear cultivar 'Doyenné du Comice', the problem of fruit setting after growth regulator application has been re-examined. GA 3, BA and some growth retardants have been used, alone or in combination.
- The experiments were done on shoots bearing a limited number of flower clusters and not on whole trees.
- GA 3 alone or in a mixture with BA could largely increase the initial fruit set in this pear cultivar.
- After June drop, however, no significant difference could be found between the treatments.
- The number of well developed seeds decreased after GA 3 treatment; this effect seemed to be reversed by BA in the 1983 experiment.
- In our experiments of two years, no significant effect of the growth retardants chlormequat and mepiquat could be demonstrated.

- The same absence of effect was recorded in 1983 by using a seaweed extract containing boron.

Plum

Webster, A.D. 1984. THE EFFECT OF PLANT GROWTH REGULATOR SPRAYS AND SUMMER SHOOT TIPPING ON THE FRUIT SET AND YIELD OF YOUNG PLUM TREES. Acta Hort. (ISHS) 149:203-210

- Sprays of GA_3 + 2,4,5-TP and/or summer shoot tipping increased the numbers of fruits harvested from young Victoria plums but had no effect on fruit size.
- Similar treatments to Marjorie's Seedling increased fruit size but had no effect on fruit numbers harvested.
- The spray treatments reduced floral-bud production on both cultivars. In the second year of treatment summer tipping again increased the numbers of Victoria fruits harvested; yields on the hormone-sprayed trees were reduced however.
- Sprays of paclobutrazol to young Grove's Late Victoria plums resulted in the abscission of all fruitlets, and this effect was only partially alleviated by supplementary sprays of GA_3 + 2,4,5-TP.

Regulation of fruit drop, parthenocarpy and fruit development

FRUIT DROP

Causes

- ♣ The fruit lost are those they have not been completely pollinated.
- ♣ Competition between the fruits for food, water, and nutrients

Fruit drop and growth regulators

- The application of plant growth regulators can re-enforce hormone balance in the peel, reducing or retarding this precocious fall and the losses at harvest (Primo et al., 1966).

- Monselise and Goren (1978) reported that the spraying of auxins prevented the dropping of fruit by maintaining the cells at the zone of abscision, preventing the synthesis of hydrolitic enzymes, such as cellulase, which decomposed the cell walls.
- Citing Riov (1974), the same authors reported that hormone balance acted on the polygalacturonase activity, which, together with cellulase, was responsible for the degradation of the two important components of cell walls, cellulose and pectin.
- The use of 2,4-D as a growth regulator to promote size and to control fruit and leaf drop was reported by Hield et al. (1964)
- According to El-Otmani (1992), the combined application of GA_3 and 2,4-D reduces the precocious drop of fruit through the action of auxin and retards the softening and senescence of the peel, by the longer harvest time, and more economical storing in areas where stocking capacity is limited and cost is high.
- Prevention of pre-mature drop of fruits: 2,4,D,IAA,IBA, 2,4,5-T, are used to prevent pre-harvest deep of sweet oranges(100 to 500 ppm)

Control of premature fruit drop in Macadamia integrifolia: effects of naphthalene acetic acid application, cincturing, and shoot-tip removal.

Williams, 2003

- The effects of NAA application, cincturing and shoot-tip removal on the incidence of premature fruit
- A single, post-anthesis application of NAA (1 ppm) increased ($P < 0.05$) the final set of macadamia fruit by 35%.
- Limb cincturing combined with shoot-tip removal increased ($P < 0.05$) initial fruit set and possibly final set.
- Cincturing alone was less effective and shoot-tip removal had no effect alone.

Application of plant growth regulators at pre-harvest for fruit development of 'PÊRA' oranges

- Almeida et al., 2004
- The treatments applied were: GA_3 + 2,4-D 12.5mg L^{-1} of each; GA_3 + 2,4-D 25mg L^{-1} ; GA_3 + 2,4-D 37.5mg L^{-1}; GA_3 + NAA 12.5mg L^{-1}; GA_3 + NAA 25mg L^{-1}; GA_3 + NAA

37.5mg L^{-1}; NAA + 2,4-D 12.5mg L^{-1}; NAA + 2,4-D 25mg L^{-1}; NAA + 2,4-D 37.5mg L^{-1}; and water (control).

- The treatments were applied 3 times, at intervals of 45 days. The variables evaluated were: rate of natural fall (%), fruit length and diameter (mm), and fresh fruit weight (g).
- None of the treatments promoted alterations in the development of the fruits, but they did reduce the natural fall rate, when compared to control, up to 78.05%, inhibiting the fruits' abscision as much as 3 months.

Effect of cobalt and silver ions and naphthaleneacetic acid on fruit retention in mango (*Mangifera indica* L.)

- *Alam et al., 2004*
- The chemicals were applied to immature fruits at pea size (5-6 mm) and again 2 weeks later when the fruit lets had developed to marble size (10-15 mm).
- Three cultivars, Sindhri, Langra (3 seasons) and Dasehari (4 seasons), were tested and it was observed that all concentrations of the chemicals significantly ($P<0.05$) increased fruit retention.
- In Sindhri, cobalt nitrate, silver nitrate and NAA treatments increased yields by 106-165%, 89-146% and 94-115%, respectively, and in Langra by 100-135%, 98-138% and 95-118%.
- Whole tree sprays of cv. Dasehari with Co(NO3)2 at 100 mg/L, AgNO3 at 100 mg/L, and NAA at 20 mg/L yielded 129%, 66% and 54% more fruit than the control.

Hormonal control of fruit growth and fruit drop in mango cv dashehari

- The gibberellin content in seed increased rapidly during early seed growth and declined as growth decreased.
- The seed was the major source of gibberellin in the fruit, the pericarp containing only traces.
- Cytokinins were present both in pericarp and seed.
- During the single period of rapid growth in fruit and seed, cytokinin concentrations increased rapidly at two periods.

- The first rapid increase in cytokinin concentrations preceds the period of rapid cell division and cell enlargement and the second increase coincides with the period of rapid cell enlargement only.
- The level of ABA-like inhibitor was high in the first 21 d preceding pollination which corresponded with the period of slow growth in fruit and heavy fruit drop.
- During the rapid period of fruit growth, the level of inhibitors decreased and that of promoters increased.
- However, in maturation and slow fruit growth period, the levels of both the growth promoters and inhibitors were low.
- Thus all the growth promoters play their role in the growth of the fruit.
- Deficiency of auxins, gibberellins and cytokinins coupled with high level of inhibitors appear to cause fruit drop in mango cv Dashehari.

Effect of aminoethoxyvinilglycine (AVG) on preharvest fruit drop and maturity of apples

- Apple trees cultivars Gala and Fuji were sprayed four weeks before commercial harvest with aminoethoxyvinilglycine (AVG), at doses of 0, 125, or 250 mg L^{-1}, and assessed for preharvest fruit drop, fruit growth, and maturation on tree.
- In 'Gala', 64 days after AVG spraying, fruit drop for control treatment was 85%, and AVG (at 125 and 250 mg L^{-1}) reduced it to 10%.
- In 'Fuji', 64 days after AVG spraying, fruit drop for control was 6%, while treatments with AVG (at 125 and 250 mg L^{-1}) increased fruit drop to 10%.
- AVG was a powerful retardant of fruit maturation for 'Gala' but not for 'Fuji'.
- In 'Gala', the most affected attribute was the skin background color, followed, in decreasing order, by soluble solids content, the starch index, skin red color, the flesh firmness, and titratable acidity. In 'Gala', only flesh firmness retention was improved by increasing AVG dose from 125 mg L^{-1} to 250 mg L^{-1}.
- The AVG at 250 mg L^{-1} inhibited "Gala" late fruit growth but not 'Fuji'.

Conclusions

- AVG substantially suppressed preharvest fruit drop and delayed fruit maturity on 'Gala' but not on 'Fuji';

- On 'Gala', the AVG dose of 125 mgL^{-1} might be used commercially to delay fruit harvest and increase yield by reducing premature fruit abscission and increasing fruit size of late harvested fruit;
- Fruit treated with AVG had poor skin color (dark-green background and deficient red color development) while still being able to mobilize starch, soften, and lose acidity on the tree.

Hormonal regulation of fruitlet abscission induced by carbohydrate shortage in citrus

- Sugar supply, hormonal responses and fruitlet abscission were manipulated through full, partial or selective leaf removals at anthesis and thereafter.
- In developing fruitlets, defoliations reduced soluble sugars (up to 98%), but did not induce nitrogen and water deficiencies.
- Defoliation-induced abscission was preceded by rises (up to 20-fold) in the levels of abscisic acid (ABA) and 1-aminocyclopropane-1-carboxylic acid (ACC) in fruitlets.
- Applications to defoliated plants showed that ABA increased ACC levels (2-fold) and accelerated fruitlet abscission, whereas norflurazon and 2-aminoethoxyvinyl glycine reduced ACC (up to 65%) and fruitlet abscission (up to 40%).
- Only the full defoliation treatment reduced endogenous gibberellin A_1 (4-fold), whereas exogenous gibberellins had no effect on abscission.
- The data indicate that fruitlet abscission induced by carbon shortage in citrus is regulated by ABA and ACC originating in the fruits, while gibberellins are apparently implicated in the maintenance of growth.
- In this system, ABA may act as a sensor of the intensity of the nutrient shortage that modulates the levels of ACC and ethylene, the activator of abscission.
- This proposal identifies ABA and ACC as components of the self-regulatory mechanism that adjusts fruit load to carbon supply

Amla

- ♣ Advances in flower and fruit regulation
- ♣ Three staged of flower and fruit drops
- ♣ Ist – highest as 70% of flower drop off with in three weeks of flowering due to degeneration of the egg apparatus and lack of pollination
- ♣ IInd – from June - Sep – due to lack of pollination and fertilization

- ♣ IIrd – drop consist of fruit of various stages beginning from third week of Aug until Oct – due to embryological and physiological factors

PARTHENOCARPY

- In botany, the formation of fruits without seeds.
- This phenomenon, of no obvious benefit to the plant, occurs naturally in some plants, such as bananas.
- It can also be induced in some fruit crops, either by breeding or by applying certain plant hormones.

GA3

- Gibberellic acid application (10 µmol pistil^{-1}) caused development similar to that in pollinated pistils, while benzyladenine (1 µmol pistil^{-1}) and naphthylacetic acid (10 µmol pistil^{-1}) treatment produced shorter siliques. Naphthylacetic acid primarily modified mesocarp cell expansion.

Auxins

- Parthenocarpy: IBA, NAA produces seed less/fruits - smaller sized fruits, but more in number, hence yield not affected.

Strong synergistic effects of gibberellins with the synthetic cytokinin N-(2-chloro-4-pyridyl)-N-phenylurea on parthenocarpic fruit set and some other fruit characteristics of apple

- The induction of parthenocarpic fruit set was investigated using the apple cvs. Golden Delicious and Jonagold.
- The gibberellins GA_3, GA_4, GA_5 and GA_7 and the synthetic phenylurea-type cytokinin CPPU (N-(2-chloro-4-pyridyl)-N-phenylurea), were applied alone and in combination to unpollinated flowers at the end of petal fall.
- Gibberellins induced only a marginal final set of parthenocarpic fruits.
- CPPU sprays were more effective, particularly in the first year.

- When applied in combination, CPPU and gibberellins had a positive synergistic effect on parthenocarpic fruit set and fruit size, but a negative effect on flower induction the next year.
- After CPPU + GA sprays, percent fruit set was similar, or greater, compared to natural pollinated trees.
- The parthenocarpic fruits induced by CPPU + GA had an increased length to diameter ratio.
- CPPU stimulated, and GA_4 and GA_7 reduced, the russeting of the parthenocarpic fruits.
- The internal quality of the fruits was hardly affected, but Ca-deficiency symptoms occurred more frequently in parthenocarpic fruits

Parthenocarpic fruit development in cloudberry (Rubus chamaemorus L.) is induced by 3b-hydroxylated gibberellins

- The purpose of this study was to test the activity of gibberellins and auxins for induction of parthenocarpic fruit development in cloudberry, Rubus chamaemorus L., a dioecious, northern wild berry
- Plant hormones were applied directly to open flowers, either dissolved in ethanol or as an aqueous spray.
- Of the tested gibberellins (GA_1, GA_3, GA_4, dimethyl-GA_4, GA_5, GA_9, GA_{19}, GA_{20} and GA_{24}) only GA_1, GA_3, GA_4 and dimethyl-GA_4 were active and induced fruit development comparable to development of pollinated fruits.
- Lack of activity of GA_{20} and GA_9, the immediate precursors of GA_1 and GA_4, respectively, suggests that 3b-hydroxylation of gibberellins can be inhibited in unpollinated flowers of cloudberry.
- Auxin treatments induced an initial fruit development, but did not result in ripe berries.
- Based on these results, use of transgenic methods to regulate gibberellin or/and auxin biosynthesis in carpels of cloudberry could be used to develop a parthenocarpic cloudberry.

Fruit set and development of three pear species induced by gibberellins

- Parthenocarpy was readily induced in two cultivars of Japanese pear when GA_{4+7} (500 ppm) in combination with or without other plant growth substances were applied on the emasculated and decapitated (stigma removed) flowers in full bloom and 3 weeks after.
- While GA_3 did not induce parthenocarpy in these cultivars.
- Gibberellin was not necessary for the second treatment (3 weeks after bloom), but could be replaced with NAA and/or BA.
- The parthenocarpic fruit was small and generally elongated-obovoid, different from the round seeded fruit.
- Parthenocarpic fruit set of European pear induced by GA_3, GA_{4+7} or GA_{45} was 42, 45 and 46%, respectively.

FRUIT GROWTH

Using urea phosphate to enhance the effect of gibberellin A_3 on grape size

- Gibberellic acid (GA_3) is widely used to enlarge the berries of seedless grapes (*Vitis vinifera* L). In cv. 'Sultana' (Thompson Seedless) the addition of 1000 mg/L urea phosphate (UP) to GA_3 solutions after fruit set reduced the pH of the solutions to a stable pH 2.9 and enhanced the effect of GA_3 on berry size and delayed maturation.
- Addition of citrate buffer, pH 2.9, to GA_3 sprays did not affect berry size or maturation.
- The possibility of improved GA penetration due to the low pH is considered.
- The nutritional effect of UP and direct enhanced penetration by the urea ion are also discussed.

Studies on apple fruit abscission and growth as affected by cytokinins

- Thinning trials were conducted for two years (1998-1999) on trees of Gala-derived cultivars ('Mondial Gala' and 'Galaxy'), using NAA and CPPU.
- In both cultivars CPPU, alone or in combination with NAA, has proven rather successful, always increasing fruit size.
- In both years and for both active ingredients the most effective concentration was 10 ppm, at various stages of fruit ontogeny.
- These applications resulted in reduced crop density (fruit number per trunk cross sectional area, TCSA) and always increased fruit size compared to hand-thinned controls.

- CPPU was also tested as a promoter of fruit growth, applied after trees had been thinned to optimum fruit load.
- Twenty ppm a.i., applied when the king fruit reached 20 mm diameter, increased fruit size, improving yield per tree.
- Earlier modelling work on fruit growth has led to the hypothesis whether CPPU's mode of action might be via a cell division stimulation, resulting in greater numbers of cells in the cortical parenchyma of the treated fruits.

A Summary of growth regulator treatments used around the world.

Growth Regulators	Effect	Timing	Concentration	Extent of Effect
GA	Reduce flower number to increase fruit size.	Early June and at bud break.	One, (or for greater effect) two sprays of 25 ppm two weeks apart. Ralex® at label rates	Proportional to crop size, variety and tree vigour. 20% reduction in flowering in Australian trials. Registration of Ralex® due 2002, restricted use trial permit currently
GA	Improve fruit set.	70-90% petal fall (October).	10 to 25 ppm.	Proportional to crop size, variety and tree vigour.
Ethephon	Thin crop load	When fruitlets are 10 to 15mm in diameter	50-70ml/100Lat about 3500L/ha	Cost effective thinning agent. Good application techniques required.
3,5,6-TPA	Thin crop load especially smaller fruitlets.	When fruitlets are 15 to 17 mm in diameter	15ppm	Proportional to crop size, variety and tree vigour.
3,5,6-TPA	Expand cell size to increase fruit size.	When fruitlets are 20 to 30 mm in diameter	15ppm	Proportional to crop size, variety and tree vigour.
2,4,-D	Expand cell size to increase fruit size.	When fruitlets are 5 to 19 mm in diameter	57-110g/ha@ 5000L/ha.	Proportional to crop size, variety and tree vigour. Isopropyl ester formulation.
Dichlorprop (2,4,DP)	Expand cell size to increase fruit size.	Mandarins: 15 to 20 mm in diameter Oranges: 22 to 25 mm in diameter	50 to 100 ppm.	Proportional to crop size, variety and tree vigour. A 4 to 5 mm increase in fruit diameter for mandarins and a 5 to 10 mm increase for oranges.
NAA	Crop thinning	When fruitlets are 15 to 20 mm in diameter	200-350ppm	Temperature dependant (rates vary to ambient temperatures)

Summary of Sprays Trialed in California (240 trees/ha)

Nutrient	Rate	Timing	Effect
Urea (low biuret)	0.5%Nat31.4kgN/ha (1.1 kg/100L low biuret urea @ application volume 6300L/ha)	Winter- Pre bloom	Increase yield without reducing fruit size. Also reported to increase TSS at harvest.
Phosphorus (Phosphite)	Nutri-phite® 0-28-26 [phosphorus & potassium mixture] at 7.3L/ha	Winter- Pre bloom	Increase yield without reducing fruit size. Also reported to increase TSS at harvest.
Urea (low biuret)	1.3%Nat31.4kgN/ha (2.8kg/100L low biuret urea @ application volume 2400L/ha)	Full bloom	Increase yield by increasing fruit set. Fruit size is not reduced.
Urea (low biuret)	1.5%Nrateat31.4kgN/ha (3.3kg/100L low biuret urea @ application volume 2100L/ha)	Maximum Peel Thickness / End of cell division (Early/Mid Summer)*	Increase fruit size
Phosphorus (Phosphite)	Two applications of Nutri-phite® at 4.6L/ha	Mid/late spring and early/mid summer*	Increase fruit size and increase TSS ratio at harvest.

Lecture No.15

Physiology of Fruit Ripening-climacteric and non-climacteric fruits-Hormonal influence

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. The solubilization may occur through an increase in methylation of the galacturonic acid or through reduction in the size of a chain of polygalacturonoid or both.

Hydrolytic changes in the fruit during ripening usually lead to the formation of sugars. 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 pigment changes occur mainly in the chloroplasts with grana into chromoplasts with loosely dispersed thylakoid membranes. Electron microscopic studies have revealed that new thylakoid membranes are synthesized in the conversion. Chlorophyll is lost due to chlorophyllase activities. 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.

Very little work has been done on development of flavour substances. In apples, numerous volatile esters, aldehydes, ketones etc. have been identified. The loss of astringent materials such as phenolics is commonly found in pomaceous fruits.

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.

The period of occurrence of climacteric peak in fruits show variation in different fruits, e.g. at the time of optimum eating quality as in pear, it slightly precedes this optimum in banana and apple or just before the fruit is fully ripe as in tomatoes. Earlier it was suggested that climacteric is associated with the hydrolysis of food reserves, but it has not been found true in all cases, e.g. orange, lemon, grapes and fig in which case climacteric rise in respiration is not found during fruit ripening. The process of fruit ripening proceeds slowly in these crops. Hence, these fruits are termed as non climacteric fruits.

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

1 Climacteric fruits,

2 Non climacteric fruits,

Climacteric Fruits	Non-climacteric Fruits
Apple	Chillies
Apricot	Orange
Avocado	Lemon

Banana	Mandarins
Sapota	Watermelon
Custard apple	Grapes
Fig	Pineapple
Mango	Strawberry
Melon	
Olive	
Peach, Pear	
Persimmon	
Plum	
Tomato	

In non-climacteric fruits, the rate of respiration remains steady during their ripening.

Climacteric rise has been found affected by low oxygen and increased concentration of carbon dioxide. Both these factors prevent climacteric rise and thus improve storage quality of fruits. Storage of fruits in polythene bags produces nearly the same effect because plastic can lower oxygen and elevate carbon dioxide around fruits.

In climacteric fruits, the respiratory climacteric initiates after the fruit growth is complete. Gane (1937) established that ethylene stimulates climacteric rise and that ripe banana stimulates it in the same manner as ethylene.

Some further researches established that there is a marked rise in ethylene formation in fruits either just at or just before the onset of climacteric rise. It has also been found that the ability of ethylene to induce climacteric rise is found in climacteric fruits.

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. They proposed that the onset of ripening is associated not only with a rise in the ability to

biosynthesize ethylene but also a marked increase in ethylene responsiveness. It has also been found that ethylene can induce a respiratory climacteric in some leaves and develop many of the pigments commonly developed in fruit ripening. In general, ethylene may be bringing about the formation of new types of enzymes in fruits.

However, ethylene is not a universal ripening hormone. Some climacteric fruits like strawberry and citrus have no effect on their ripening by the ethylene treatment.

Leopold and Kriedemann (1975) concluded that ripening appears as an unveiling enzyme system which brings about the alteration of the fruit, and respiratory energy must be provided both for the synthesis of the new enzymes system and for their actions in ripening. Hormonal regulation may be involved in the change from a mature fruit resistant to a ripening to one which becomes receptive to ripening signals.

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 from chloroplasts which are rich in green pigment **chlorophyll** into **chromoplasts** which are rich in red or yellow **carotenoid pigments.**

Carotenoids are important constituents of chloroplasts and are present in green fruit tissues even before maturation. Maturation does not always involve accumulation of carotenoid pigments. For instance, yellowing in many varieties of apples, pears, grapes, olives and mature bananas results from pre-existing carotenoids which are unmasked due to disappearance of chlorophyll.

A large number of other fruits such as citrus, tomato, capsicum etc., accumulate large amounts of carotenoids which are biosynthesized during later stages of maturation. The complement of carotenoid pigments in these fruits, however, differs greatly from one species to another. In tomatoes, the carotenoid pigments are dominated by **lycopene** and β-**carotene.**

(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.

Lecture No.16

Physiology of seed development and maturation

The embryo, or embryonic plant, is the beginning of a new generation. A reserve of stored food, either as cotyledons attached to the embryonic axis or as endosperm tissue, functions as an initial source of nourishment for the embryonic plant until it attains an independent autotrophic existence. The seed coat serves to protect the embryo against adverse environmental conditions and, in some cases, is adapted as a means of seed dispersal. The early developmental stages of the embryo sac are nourished by the cells of the surrounding ovulary tissue. These cells are originally rich in starch, lipids, and proteins, which are subsequently hydrolyzed to form soluble sugars, amino acids, organic acids, and other metabolically active materials. The ovule is also connected to the main transport system of the plant by a vascular strand through which water, ions, and the other solutes are supplied to the developing seed.

The diploid nucleus of the zygote receives one chromosome complement from the female parent (Egg) and one from the male parent (sperm). Thus the *zygote* contains all the genetic information necessary for development of a mature plant. It is obvious, however, that the cells in a seedling or mature plant are not alike and that these differences arise very early in the life of the plant. The first division of the zygote forms two cells, which are generally different in size. Further divisions of these cells lead to embryonic development and the differentiation of tissues and organ systems characteristic of the plant.

Endosperm development and composition

Endosperm tissue is composed of cells with three chromosome sets (3n), two from the maternal and one from the paternal parent. This is the situation encountered in many plants, but in the gymnosperms, such as pine and hemlock, the functional equivalent of the angiosperm endosperm has a different chromosome complement and is derived from the female gametophyte, which is composed of haploid (1n) cells. Regardless of its origin and chromosome number, the endosperm serves a very special function in nourishing the embryo during early seed formation and maturation and later during seed germination before the embryo develops into an independent plant.

In angiosperms where the endosperm is commonly in the triploid (3*n*) condition, the development of the endosperm generally precedes the development of the zygote. That is, even though the fusion of the egg and sperm nuclei form the zygote (2*n*) and the fusion of the sperm nucleus with the polar nuclei form the endosperm nucleus which may occur simultaneously, the 3n endosperm nucleus usually divides to form numerous nuclei before the zygote begins to divide. Frequently the endosperm develops in the free nuclear condition without forming cell wall materials so that a liquid endosperm containing many free nuclei results. Coconut milk is an example of such a liquid endosperms during the early stages of seed development. Probably the endosperm of many other plants passes through a similar free-cell liquid stage. During later stages of endosperm development, cell walls form and a cellular, or solid, endosperm is produced. In many dicotyledonous plants the endosperm is absorbed by the cotyledons of the developing embryo. The food reserves of the cotyledons serves as a nutrient source during germination. In other plants, particularly monocots (maize, wheat, etc), the solid endosperm persists and becomes a part of the seed, where it functions to nourish the developing embryo during seed germination.

Morphological and biochemical changes accompanying seed development

The morphological aspects of embryo development (embryogenesis) following pollination, fertilization, and zygote formation have been described for many plant species. Biochemical changes accompanying embryogenesis and seed development are characterized by vigorous anabolic processes, resulting in the formation of new cells, tissues and organs rich in proteins, nucleic acids, carbohydrates and fats. The early stages of seed development, Phase I, Figure 16-2, involve pollination, fertilization, and zygote formation, processes that contributes very little to dry weight formation but must involve intense metabolic activity. Much more is known of the biochemistry of embryogenesis, initiated by cell division of the zygote. The embryo increase in dry weight as new cells are formed and cellular constituents synthesized. This is a period of intense metabolic activity with a high demand for low molecular weight precursors, such as sucrose, amino acids, fatty acids, nucleosides, organic acids, water, and inorganic ions. The bulk of these materials are supplied by the parent plant through vascular connections, but some also comes from the dissolution of cellular material in the ovule and embryo sac. Phase I comes to an end when the embryonic plant is fully differentiated and cell division ceases.

Full-term embryos, excised and nourished by a suitable array of organic molecules and inorganic ions, will continue to develop and form mature plants. The young embryos generally require growth substances in addition to organic and inorganic nutrients. The developing seed is in direct vascular contact with the parent plant. If environmental factors, such as low or high temperature, reduced light, moisture stress, or mineral deficiency, alter the metabolism of the parent plant, the pattern of development during embryogenesis may be altered.

Precocious germination is prevented by the action of an inhibitor from ovule tissue. The inhibitor, abscisic acid, may move into the ovule through vascular connections from the parent plant or it may be synthesized in the ovule. Abscisic acid prevents premature or precocious germination. Later in seed development, when vascular connections between the ovules and parent plant are broken by desiccation, low seed water content prevents premature germination.

Phase I comes to an end when the embryonic plant is fully formed and cell division ceases. Seed dry weight continues to increase rapidly during Phase II, however, because of the synthesis and deposition of seed storage materials-starch, protein, fats, phytin, etc- in endosperm or cotyledonary tissues. In monocots such as maize and rice, the endosperm cells lose their nuclear material and fill up with starch and phytin. A specialized tissue, the aleurone layer, forms to the outside of the endosperm, next to the developing seed coat. The aleurone layer may be several cells thick and is composed of dense living cells filled with protein. In dicots, such as pea, the cotyledonary cells retain their nuclear contents and become packed with starch, protein lipids, and phytin.

Phase II is the period of maximum seed dry weight increase. The storage materials are synthesized from small precursor molecules from the parent plant. The synthesis and deposition of storage molecules in developing seeds constitute a major sink for carbohydrate and nitrogenous components made by the parent plant. Sucrose, the major product of leaf photosynthesis, supplies carbon skeletons for starch and fats. Moreover, sucrose is a source of carbon skeletons for nitrogenous constituents – amino acids, amides, nucleotides. During seed filling, the demand for carbonaceous and nitrogenous molecules is high and may not be met by current CO_2, NO_3, or N_2 assimilation. In such cases, reserve materials in the parent plant may be mobilized and transported to developing seeds. To obtain maximum seed yields, especially in food plants such as maize, peas, soybeans, and beans, it is essential that the leaves and other

assimilatory organs of the parent plant be kept active as long as possible. In soybeans, it has been observed that leaf nitrogenous compounds have been hydrolyzed and transported to developing seeds under conditions when the roots cannot supply enough nitrogenous material to support seed filling. The loss of leaf nitrogen leads to premature leaf senescence and the loss of photosynthetic surfaces for carbon assimilation.

Phase II comes to an end as the seed begins to lose water. The synthesis of storage molecules involves the elimination of water molecules, but there appears to be an accelerated process of water loss, possibly through an alternation of membrane structure. Vascular connections between the developing seed and parent plant are broken so that no water or solutes can move into the seed. Moisture content during seed filling may be in the range of 50 to 60%, but after the desiccation process is under way, water content drops to 10 to 15% at maturity. Water loss is not uniform in all parts of the seed. The embryonic axis, composed of nonvacuolated parentchyma cells, contains relatively little free water, but the structural components are hydrated. Cells in endosperm and cotyledonary tissues, however, contain low amounts of water. Also, the tissues surrounding the seed that develop into seed coats undergo desiccation and sclerification, forming a hard protective structure.

During Phase III, the desiccation process continues, attaining moisture levels of between 5 and 15% (total seed). With desiccation, the subcellular organelles in cotyledonary cells seem to lose their structural integrity. In addition, organized ribosome's (polysomes) essential for protein synthesis break up into single ribosome's. The entire picture is one of very low metabolic activity, and if seed moisture remains low, further development of the embryonic axis into a mature plant does not occur. The seed is said to be dormant.

To obtain seeds of high vitality and vigor, it is important that seed moisture levels be brought to at least 10 to 12% during maturation. In some instances, it is necessary to dry seeds by artificial heat if they are harvested early. Several hybrid maize varieties, for instance, have been developed for regions with short growing seasons. If the growing season is terminated early by frost and cold weather, the normal seed desiccation process may not bring the seed moisture levels sufficiently low for safe storage. The grain is harvested and then put through a slow drying process to attain moisture levels of around 10% or lower. This process is costly and adds to the expense of producing the crop. If the grain is not dried, it may spoil in storage by fermentive

respiratory processes or by fungal and bacterial growth. In the dry state seeds can withstand environmental conditions generally unfavorable to growth: Low temperature, drought, excessive water, fire, and toxic materials in the soil.

Commercial seed producers, especially flower seed growers, follow rather stringent procedures of seed drying, storage, and packaging so as to provide the home gardener with a quality product – seeds that will readily germinate to produce a vigorous plant.

Lecture No.17

Dormancy of Seeds and Buds and Germination

Dormancy of seeds

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 of seeds.

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.

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, O_2 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 O_2 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 α-amylase and β - 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 α-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 β - 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.

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 on 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.

Ex.No.1. Estimation of photosynthetic pigments

Chloroplasts are dynamic entities of green cells in which photosynthetic processes take place. The light energy absorbed by the chloroplast is converted into chemical energy and starch is synthesized utilizing CO_2 and H_2O as raw materials and releasing O_2 as byproduct. The whole sustenance of a plant depends on the green pigments present in the chloroplast. The chloroplast is a double-layered membrane, consists of stroma (internal matrix), which is colourless, and grana which is green in colour. Photosynthetic pigments are localized only in thylakoid membrane.

Chlorophylls and carotenoids are the main pigments that take part in photosynthesis. There are several types of chlorophylls which differ from one another in the details of their molecular structure and the absorption properties. Most common chlorophyll types in the vascular plants are chlorophyll a and b. Chlorophyll a is found in all photosynthetic eukaryotes, which converts light energy into the chemical energy. Chlorophyll a is a large molecule with a tetra pyrrol ring and a magnesium ion held in it. Attached to one of the rings is a long insoluble hydrocarbon ring, a 20-carbon phytol group. Chlorophyll b has a -CHO group in the third carbon of second pyrrol ring instead of $-CH_3$ group as in the case of chlorophyll a.

Chlorophyll a and chlorophyll b have typical absorption spectra of solar radiation. Maximum peak of chlorophyll a is observed in blue violet (429 nm) and in red region (660 nm) while the chlorophyll b absorbs at 453 nm and 642 nm in ether extracts. Chlorophyll a is usually blue green and chlorophyll b is yellow green in colour. The empirical formula for the chlorophyll a molecule is $C_{55}H_{72}O_5N_4Mg$ and chlorophyll b molecule is $C_{55}H_{70}O_6N_4Mg$. The method of Arnon (1949) is employed in the estimation of the chlorophyll pigments.

Another class of accessory pigments involved in the absorption of light energy during photosynthesis is the carotenoids. Carotenoids are 40-carbon compounds. They are the red, orange, or yellow fat-soluble pigments found in chloroplasts. Normally two kinds of carotenoids are found in chloroplasts. They are carotenes and xanthophylls. Xanthophylls contain oxygen in their molecules whereas carotenes do not.

Estimation of Chlorophyll content

Reagent required

80% Acetone

Procedure

250 mg of leaf sample is macerated with 10ml of 80% acetone using a pestle and mortar and the extract is centrifuged at 3000rpm for 10 minutes. The supernatant solution is transferred into a 25ml volumetric flask and made up to 25ml using 80% acetone. The color intensity of the green pigment is read at 645nm, 663nm and 652nm for chlorophyll a, chlorophyll b and total chlorophyll content respectively using Spectrophotometer.

Formula for calculation

$$\text{Chlorophyll a} = 12.7\,(\text{OD at } 663) - 2.69\,(\text{OD at } 645) \times \frac{V}{1000 \times W}$$

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

$$\text{Total chlorophyll} = \frac{\text{OD at } 652 \times 1000}{34.5} \times \frac{V}{1000 \times W}$$

Where

OD - Optical Density

V - Final volume of supernatant (25ml)

W - Fresh weight of the sample taken in gram (0.25 g)

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

$$\text{Chlorophyll a and b Ratio} = \frac{\text{Chlorophyll a}}{\text{Chlorophyll b}}$$

Ex.No.2. Estimation of photosynthetic enzyme

Soluble protein

Wildman and Bonner (1947) found that leaves contain a soluble protein, which they called **Fraction I** protein. Growing and expanding leaves of many species of plants were found to contain this protein, which subsequently was found to be identical to RuBP carboxylase (RuBP Case) enzyme. This enzyme is present in all plants containing chlorophyll *a* as well as many photosynthetic algae and bacteria. RuBP Case has been isolated and purified from the leaves of many plants and in 1971 the protein was crystallized from tobacco leaves in Wildman's laboratory . The protein occurs in stromal fraction of chloroplast at concentration as high as 300mg ml^{-1}. Crystalline RuBP Case has a molecular weight of 557, 000 and is composed of eight large sub units (MW 55800 each) and eight small sub units (MW 12000 each). The large sub units are synthesized within the chloroplast, whereas the small sub units are synthesized outside the chloroplast in the cytosol. The small sub units then penetrate the chloroplast envelope and the complete protein (RuBp case) is assembled in the stroma.

RuBP case is known as the most abundant protein in the world because of its widespread occurrence in plants and its relatively higher concentration in the soluble protein fraction of leaves (>70%). RuBP Case enzyme is of special importance because it catalyses the addition of CO_2 to Ribulose 1-5 bisphosphate with the formation of 3PGA. Hence, the estimation of soluble protein fraction containing RuBP carboxylase will be enabling to study the rate of CO_2 fixation and thereby provide information concerning the relative amount of CO_2 fixed by different species of plants.

Principle

The blue colour was developed by Biuret reaction of the protein with the alkaline copper tartarate in which phosphomolybdic and phosphotungstic components in the Folin- Ciocalteau reagent are reduced by the amino acids tyrosine and tryptophan respectively present in the protein. The colour intensity is measured at 660 nm.

Reagents required

1. **Phosphate buffer**

   Monobasic ($NaH_2 PO_4$) – 0.2 M

   Dibasic (Na_2HPO_4) – 0.2 M

   16 ml of Monobasic + 84 ml of Dibasic mixture is made up to 200ml with distilled water.

2. **Alkaline Copper Tartarate reagent (C)**

   2% sodium carbonate in 0.1 N sodium hydroxide (A)

   0.5 % copper sulphate (Cu $S0_4$ $5H_20$) in 1 % sodium potassium tartarate (B)

   Mix 50ml of A and 1ml of B prior to use

3. **Folin - Ciocalteau reagent (1:2 with water)**

Procedure

Leaf sample of 0.25 g is macerated with 10ml of phosphate buffer solution and the leaf extract is centrifuged at 3000rpm for about 10 minutes and the supernatant solution is collected. One ml of the supernatant solution is pipette out into a test tube and 5ml of Alkali Copper reagent is added. The solution is kept as such for 30 minutes for color development. Then, 0.5ml of phenol reagent is added and the OD value of the sample is read at 660nm in Spectrophotometer. The soluble protein content is expressed as mg g^{-1} of leaf sample.

Standard

50mg of Bovine Serum Albumin (BSA) dissolve in 100ml of distilled water, which gives stock solution of 500ppm. Prepare different concentrations of BSA standard solutions like 100, 200, 300, 400 and 500ppm by diluting the stock solution. Run the series of standard in similar way as that of your sample and draw a standard graph. Plot your sample OD in the standard graph and find out the corresponding concentration (x

μg). From that, calculate the amount of soluble protein present in the given sample by using following formula.

Calculation

$$\text{Amount of soluble protein} = \frac{X}{0.5} \times \frac{25}{500} \times 1000$$

Amount of soluble protein present in the given sample is expressed in mg/g.

Ex.No.3. Measurement of leaf area following various methods

Leaf is assigned as one of the important organs of plant system and further development of plant depends upon the persistence of leaves. Physiologically, leaf area constitutes the main photosynthetic surface and supplies most of the photosynthates required by the seed, fruit or any storage organs. So the estimation of leaf area is an essential integral part of classical growth analysis and is often important in physiological reasoning of variations in crop productivity. For the estimation of leaf area, several methods have been developed. 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. So this type of method cannot be used for estimating the leaf area for all types of leaves. For estimating the leaf area, the outline of the leaf is drawn on a graph paper and the number of full squares, half squares and quarter squares are counted and added. The leaf area is expressed as cm^2 per leaf.

2. Electronic method (leaf area meter)

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

the image of leaf on the mirror and the reading is measured digitally. Area of leaf is expressed in cm^2.

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.

4. Weight basis method

The leaf area (L_1) occupied by known dry weight (W_1) 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 (L_2) based on the total dry weight (W_2) by using the given formula.

$$\text{Whole leaf area (L2)} = \frac{L_1}{W_1} \times W2$$

Where, L_2 - Total leaf area;

L_1- Single leaf area,

W_1 – Single leaf weight (dry)

W_2 - Total leaf weight (dry)

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

Ex.No.4. Growth analysis

Growth is a characteristic of life. It is the foremost symbolization of life in action. Growth continues till the end of an organism. It is expressed as height, weight (size), volume, number and area. Growth is always phenomenal. If growth stops vertically it may commence horizontally. If leaf expansion stops, it starts gaining in number and volume. Thus the growth is multifaceted, remarkable accomplishment of life. The shape and size of an organism are because of its continued cellular development and differentiation. Practically, every cell undergoes sequential phases such as juvenile, grand growth and senescence. This is a sigmoidal function. Growth rate is quantified mathematically in terms of time as in cases of other vital processes of plants and this gives us a valuable information documenting growth as influenced by various abiotic, biotic, edaphic and seasonal factors.

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.

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

$$W_1 = W_0.e^{rt}$$

Where, W_1 = the final size (weight, height, etc.,) after time't'.

W_0 = the initial size at the beginning of the time period

'r' **=** the rate at which plant substance is laid down during time ***'t'***

'e' **=** the base of natural logarithm.

The above equation also indicates that the size of an organism ($\mathbf{W_1}$) depends on the initial size (**Wo**). The plant size also depends on the magnitude of ***r*** (relative growth rate). 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 and called ***'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.

PARAMETERS OF GROWTH ANALYSIS

1. Leaf Area Index (LAI)

It is the leaf area per unit area of land occupied by a plant.

$$LAI = \frac{\text{Leaf area per plant}}{\text{Land area occupied by a plant (spacing)}}$$

Watson (1956) suggested this formula. LAI is the measure of available photosynthetic surface per unit land area.

2. Leaf Area Duration

It is the ability of the plant to maintain the green leaves over unit area of land throughout its life. 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 employed the formula

$$LAD = \frac{LAI\ (i) + LAI\ (ii)}{2} \times t_2 - t_1$$

LAI (i) - Leaf area index at first stage

LAI (ii) - Leaf area index at second stage

t_2-t_1 - Time interval between the two consequent stages and expressed in **days**.

3. 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 $\mathbf{cm^2\ g^{-1}}$

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

In broad sense, LAR represents the ratio of photosynthesizing to respiratory material within the plant.

4. Specific Leaf Area (SLA)

It is the ratio of assimilating area to its dry weight. It was suggested by Kvet ***et al.*** (1971) and the formula used to calculate SLA is

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

It is expressed as **cm^2 mg^{-1}.**

5. 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}.**

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

6. LEAF WEIGHT RATIO (LWR):

It is the ratio of leaf dry weight to the plant dry weight. It is the measure of leafiness of the plant on a weight basis. It is expressed in **g kg^{-1}**

$$LWR = \frac{\text{Leaf dry weight}}{\text{Plant dry weight}}$$

7. Net Assimilation Rate (NAR)

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

$$NAR = \frac{\text{Loge } L_2 - \text{Loge } L_1}{L_2 - L_1} \times \frac{W_2 - W_1}{t_2 - t_1}$$

Where

Log e L_2 = Natural log of leaf area at stage 2

Log e L_1 = Natural log of leaf area at stage 1

L_2 & L_1 = leaf area at stage 2 & 1 respectively.

W_2 & W_1 = Dry weight of the whole plant at stage 2 & 1 respectively

$t_2 - t_1$ = Time interval between the two stages

NAR is expressed as **mg cm^{-2} day^{-1}**

8. 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.

$$RGR = \frac{LogeW_2 - LogeW_1}{t_2 - t_1}$$

W_2 & W_1 = Whole plant dry weight at t_2 & t_1 respectively and expressed as **mg g^{-1} day^{-1}**

9. Relative Leaf Growth Rate (RLGR)

It is the difference in leaf dry weight over a period of time. Fisher (1921) suggested the formula and the values expressed as **mg g^{-1} day^{-1}.**

$$RLGR = \frac{LW_2 - LW1}{t_2 - t_1}$$

RLGR involves consideration of physiological activity like photosynthesis, respiration, nutrient uptake and metabolic balance.

10. 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.

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

Where

W_2 and W_1 – are plant dry weight at time intervals t_2 and t_1

P-population per unit area

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

CGR = LAI x NAR

CGR increases as LAI increases because of greater light interception.

CGR is expressed as **g m^{-2} day^{-1}**

Crop = Soybean; Spacing = 30x10 cm^2

CASE STUDY

Total dry matter production (g)

Treatments /DAS	15	30	45	60	75
Control	0.225	1.04	3.79	9.50	20.98
KH_2PO_4 (1%)	0.239	1.17	4.40	11.70	33.78
Urea (1%)	0.230	1.14	4.14	11.28	30.57
Mepiquat chloride (125 ppm)	0.210	1.07	4.02	10.25	26.18

Leaf dry weight (g)

Treatments /DAS	15	30	45	60	75

Control	0.140	0.810	2.50	4.20	6.00
$KH_2 PO_4$ (1%)	0.140	0.810	4.25	5.98	7.94
Urea (1%)	0146	0.892	4.15	5.69	7.79
Mepiquat chloride (125 ppm)	0.149	0.820	3.81	5.20	7.27

Leaf area (cm^2)

Treatments /DAS	15	30	45	60	75
Control	679	1132	1451	1814	1620
$KH_2 PO_4$ (1%)	850	1536	1996	2495	2170
Urea (1%)	927	1686	2134	2668	2320
Mepiquat chloride (125 ppm)	762	1385	1753	2192	1940

Ex.No.5. Bioassay for hormones

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 mm^3) 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 (α) 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.

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 GA_1 in dwarf corn (strains d_1, d_3 and d_5) is in the following order:-

$$d_1 - GA_1 > GA_3 > GA_4 > GA_7 > GA_5 > GA_6 > GA_8 > GA_9$$

$$d_3 - GA_5 > GA_7 > GA_9 > GA_3 > GA_4 > GA_1 > GA_6 > GA_2$$

$$d_5 - GA_5 > GA_7 > GA_3 > GA_4 > GA_9 > GA_1 > GA_2 > GA_2 > GA_8$$

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

Dwarf Corn Test

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.

Bioassay for kinetin (or 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.

- The classical methods for bioassays of plant growth hormones auxins, gibberellins etc have now been replaced with modern methods of separation and quantification such as high performance liquid chromatography (HPL C) and gas chromatography (GC) which are then followed by mass spectrometry (MS) to provide proof of structure. Immuno-assay methods are also used which are more rapid and thousand times more sensitive than bioassays.

Ex.No.6. Preparation of hormonal 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.

Example

To prepare a 10 per cent $CuS0_4$ solution, we add 10 g of $CuS0_4$ 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.

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.

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 H_20.

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 (H_2S0_4) having a mol. wt. of 98, has 2 replaceable hydrogen atoms. Therefore a normal solution of H_2S0_4 contains 49 g (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 K2SO4 contains 174.26/2 = 87.13 g/liter (K_2S0_4 contains 2 hydrogen equivalents).

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.

Example: 1 M sucrose: 342.2 g sucrose dissolved in a small quantity of water and the volume is made up to 1 liter.

342.2 mg dissolved in liter - 1 mM 342.2 u g dissolved in liter

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.

Eg: 1. 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.

2. K_2S0_4 - Mol. wt. = 174.2

174.2 g in a liter = 1 M K2SO4

To get 1 M of K the amount of K_2S0_4 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.

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.

Ex.No.7

Assessment of hormonal influence on induction of rooting in cutting

The most common use of plant regulators in horticulture is to induce root of cutting in many horticultural plants. The most commonly employed compound is indole butyric acid (IBA) which is the most effective one and the other compounds are Naphthalene acetic acid (NAA). Indole acetic acid (IAA) and 2,4-Dichloro phenoxy acetic acid (2,4-D). Certain of these chemicals can be used in a powder form mixed with talc. The cuttings may be moistened with water at their lower ends and then dipped in the powder and planted, afterwards, lower end going beneath the soil. Some patented products like Saradix A and B can be used in the form of powder. The lower end of the cuttings may be soaked in such solution of low concentrations like 10,25,50 parts per million (ppm) for 18 to 24 hours (soaking method) or at higher concentration from 500 to 2000 ppm for a minute or less (quick dip method). The concentrations differ according to the type of cuttings viz herbaceous, semi hardwood and hardwood cuttings. Another usage of plant regulators in propagation of plant is in rooting of air layering. Gibberellic acid is one such plant regulator which is used to induce vigorous growth of nursery plants. When this chemical is applied to the growing tips, this chemical will induce rapid cell division and cell elongation resulting in very rapid growth.

Method of application of growth regulators

1. **Powders:** Woody and difficult to root species should be treated with higher concentrations of preparations whereas tender and succulent and easily rooting species should be treated with lower concentrations. Fresh cuts should be made at the base of the cutting shortly before they are dipped into the powder. The powder adhering to the cutting after they have lightly tapped is sufficient.

Talc preparation has advantages of being readily available and easy to use, but uniform results may be difficult to obtain owing to variability in the amount of material adhering to the cuttings.

2. **Dilute solution soaking method**: The basal part i.e. 12.5 cm of cutting will soak in a dilute solution of the material for about 24 hours just before they are inserted into the rooting media. The concentration may vary from 20 to 200 ppm.

3. **Concentrated solution dip method**: In the dip method, the concentrated solution vary from 500-10000 ppm of root promoting chemical in 50% alcohol is prepared and the basal 1 -2 cm of cutting are dipped in for a short time about 5 sec. Then the cuttings are inserted in the rooting media. Here, more uniform results are likely to be obtained. Once prepared and used chemical should be thrown instead of reusing. 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 (*Vitis vinifera)*	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%)	Higher rooting percentage
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 (large red, large yellow and Alpha)	Stem cuttings	IBA	Recommended for commercial propagation
Citrus (C.aurantifolla)	Air-layers	IBA (1.0%) and IAA (1.0%)	Increased rooting
Guava	Air – layering	IBA (250mg/l in lanolin)	Significant improvement in the rooting

Ex. No. 8. Assessment of hormonal influence on control of flower and fruit drop

Regulation of flower drop and thinning in fruit crops

Thinning is done for two reasons.

- First, a certain portion of the fruit is removed, so that the remainder will develop adequate size and quality.
- Second, the thinning process serves to increase the plant's ability to form flower buds for the next year.
- Thinning also reduces the total load on the branches and reduces breakage.
- Thinning is necessary for apples, nectarines, pears, plums, and peaches.

Thinning the fruit will provide many advantages

- Fruit Size - the resulting fruits will be much larger.
- Return Bloom - next year the tree has a much better chance of producing a decent crop, as opposed to developing a habit of biennial bearing.
- Tree Stress - with a lighter crop (fewer fruit) the tree is under less stress.
- Flavour is improved with a lighter crop

Chemicals used for thinning

NAD

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

Benzyladenine (BA)

- Is marketed under the trade name Accel.

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

Carbaryl

- Reducing the number of fruits per cluster.
- It has been shown to be effective in increasing fruit size but has no hormonal effect in increasing return bloom.

NAA

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

Grapes

Effect of crop load, girdling and berry thinning on water berry development in grapes.

Suneel Sharma *et al*., 1999

Hisar during 1995, on 9-year-old vines of cv. Perlette.

Treatments

T_1 - Trunk girdling (80 bunches / vines)

T_2 - Berry thinning (80 bunches / vines)

T_3 - Crop load of 40 bunches / vine

T_4 - Crop load of 60 bunches / vine

T_5 - Crop load of 80 bunches / vine

- Berry thinning was done at berry set stage by removing the lower one third portion of main stem of each cluster leaving 12 laterals at the base of the cluster

Results and discussion

- Minimum incidence of 7.02% was observed in treatment involving crop load of 40 bunches / vine followed by berry thinning.
- The decreased crop load to 4o / 60 bunches vine ensures balanced partitioning of photosynthetic assimilates to the developing bunches, there by enabling the vine to meet the nutritional requirement.
- Increased bunch weight (4 and 7.50g) could be attributed to the effect of girdling and cluster and berry thinning treatments increased the amount of CHO to the growth and development of the bunches.

Peach

- The no of leaves to be retained per fruit after thinning is 30-40

Teskey and Shoemkar, 1972

- Fruits were thinned at 15 to 20cm apart from each other
- In low chilling cvs. 10-15cm

Singh, 1969

Chemical thinning

- 300 ppm 3- CPA
- 2-3 % thiourea
- 40-100 ppm 2, 4, 5-T
- 50ppm 2, 4-D

Erez, 1975

Time of thinning

- Before first fruit drop, even blossom thinning is more effective than late thinning
- Redhaven – 35-45 days after full bloom/when fruits are 10-15 mm dia

Lekson, 1981

Evaluation of the effectiveness of Armothin for chemical peach thinning.

Tsipouridis and Thomidis, 2005

- Fruit set on 'Andross' trees sprayed with 3% Armothin were lower than on those sprayed with 1.5% Armothin.
- Both 3 and 1.5% Armothin resulted in less fruit set than in the untreated control.
- The rootstock used did not influence the effectiveness of Armothin.
- However, when treated with 3% Armothin, the number and proportion of fruit set in the cultivar 'Flavour Crest' was much higher than that set in the cultivar 'May Crest'.
- The highest percentage of fruit set per annual shoot ranged from 10 to 40% treated with 3% Armothin,
- Whereas the range was 60–80% on the untreated control.

Heading fruiting shoots before bloom is equally effective as blossom removal in peach crop load management.

- Marini, 2002
- Three experiments were performed in Virginia,' USA, in 1993, 1995 and 1996.
- Year-old shoots on 'Cresthaven' trees were headed by 50% or blossoms were removed from the terminal half of each shoot.
- At 45 days after full bloom, all trees were hand thinned to obtain predetermined crop densities.
- The average fruit weight was highest on trees with blossom removal. But crop value and net profit was highest for nontreated trees.

- Fruit weight and crop value were not affected by the percentage of shoots treated.
- Heading of shoots reduced fruit set, number of fruits removed at thinning, and thinning time per tree.
- Yield, crop density, and average fruit weight were not affected by heading.
- Profit was increased by shoot heading in I of the 3 years.
- Results from this study indicate that heading peach shoots by 50%, while dormant pruning can reduce thinning costs without reducing fruit size.

Partial flower thinning increases shoot growth, fruit size, and subsequent flower formation of peach.

- Myers *et al*., 2002
- In field experiments conducted in Virginia, USA
- Cultivars Redhaven (USA) and Golden Queen (New Zealand) during bloom to 50% of the necessary level by hand, followed by adjustment hand thinning at 42 days after full bloom (DAFB), was compared to a similar degree of thinning accomplished entirely at 42 DAFB by hand.
- Compared to unthinned trees and trees thinned at 42 DAFB, partial flower thinning increased the subsequent development of flower buds per shoot and the number of flower buds per node.
- The number of flower buds on the proximal five nodes of shoots 15.0-30.0 cm in length was increased, although not on shoots 5.0-7.0 cm in length.

Peach fruit weight, yield, and crop value are affected by number of fruiting shoots per tree

- Richard and Marini. 2003
- About 40 days after bloom each year, fruits on all trees were thinned to similar crop loads, so only the number of fruits per shoot varied.
- Fruit set and number of fruits removed by hand thinning were positively related to number of fruiting shoots retained per tree.

- Number of fruits harvested per tree was not related to number of shoots per tree, whereas average fruit weight at thinning and at harvest, and crop value per tree were negatively related to the number of shoots retained per tree.
- The number of fruiting shoots retained per tree during pruning should be one-fifth to one-seventh of the number of fruits desired per tree, so that five to seven fruits per fruiting shoot are retained after hand thinning.

Plum

Fruit Thinning

- Removal of parts of the crop enables the remaining crop to receive a greater share of the food manufactured by the leaves.
- Thinning also results in removal of the undesirable fruits and thus reduces limb breakage.
- Early thinning tends to increase average fruit weight and hasten maturity without affecting total yield (Belmans and Keulemans, 1987).
- Wells and Bukovac (1978) reported that fruit thinning significantly increased fruit size, soluble solids and colour, and markedly decreased yield in Stanley plum.
- Batjer (1965) suggested a spray of 4, 6-dinitro-ortho-cresol (DNOC) 42 to 85 ml per 100 litre,
- Other chemicals that have been suggested for use as plum fruit thinners are 2, 4, 5-trichlorophenoxy acetic acid with or without gibberellic acid (Paunovic and Ogasonovic, 1979), naphthaleneacetic acid (Lat and Thakur, 1979), 3-chlorophenoxypropionie acid or 3-chlorophenoxy propionamide or a mixture of both (Webster, 1980; Beutel, 1969) and (2-chloroethyl) phosphonic acid (Martin, *et al.,* 1975).
- The spray should be applied slightly before full bloom to obtain adequate thinning.

- In case inclement weather occurs during the pre bloom period, which could quantitatively affect the full bloom and subsequent fruit set, the spray may be delayed until a day or two after full bloom.
- Lupescu and Tertecel (1985) in trials with 11 year old Stanley plum, sprayed FIordimex (ethephon) at 200 or 400 ppm or NAA at 20 or 30 ppm, 2 weeks after full bloom.
- They found that ethephon at 200 ppm produced yields of 28-29 tones/ha compared with 23-27 tones/ha in the control.

Effect of thinning and of trunk girdling on growth, production and quality of Japanese plums.

- Ilha *et al*., 1999
- The effect of manual fruit thinning (0, 25, 50 and 75%) and trunk girdling (4 mm width) on the growth, production and quality of Japanese plums cv. Amarelinha) was evaluated.
- Thinning was performed on 16 October 1995, 36 days after full bloom, and girdling at 7 days after thinning.
- Thinning decreased total fruit yield proportionally to the applied thinning intensity.
- Trunk girdling decreased fruit firmness, titratable acidity and increased the ratio of total soluble solids: titratable acidity,
- But decreased the vigour and vegetative growth of the trees.
- None of treatments significantly affected the mean fruit weight.

Apple

Thinning Golden Delicious apples with pre-bloom naphthalene acetic acid and later carbaryl sprays

- Jones *et al., 2005*

- The NAA was applied at pink bud or balloon blossom at 12, 18 and 24 ppm, with or without carbaryl at 1800 ppm applied 10 d after full bloom.
- The higher concentrations of NAA did the most thinning
- But only the hand-thinned and 24 ppm NAA at balloon blossom with carbaryl treatment thinned enough to produce the required fruit size.
- The NAA sprays at balloon blossom thinned considerably more than those at pink bud.
- Neither NAA nor carbaryl alone was as effective as the combination.

Studies on the use of 2-chloroethyl-phosphonic acid (ethephon) as a thinning agent for apples.

- Veinbrants*, 2005*
- 2-chloroethylphosphonic acid (ethephon) at concentrations from 50 ppm to 400 ppm was applied at various stages during and after flowering to apple cultivars Golden Delicious, Gravenstein, Jonathan, Richared and Starkrimson.
- On the heavy setting Golden Delicious, Gravenstein and Jonathan cultivars, adequate thinning and satisfactory fruit size was obtained when ethephon at 100 ppm was applied at or shortly after full bloom, followed by naphthalene acetic acid.
- On the lighter setting Richared and Starkrimson cultivars, one ethephon spray at 100 ppm applied at or shortly after full bloom resulted in adequate thinning.
- All fruit were eliminated on Jonathan, Richared and Gravenstein cultivars when ethephon was applied at 400 ppm, 300 ppm and 200 ppm, 36, 35 and 42 days after full bloom, respectively.

Combining ethephon and naphthalene acetic acid (NAA) in one spray to thin `Golden Delicious' apples

- Jones *et al*., 2000
- All sprays were applied only at full bloom (FB).

- Ethephon sprays of 0, 50, 100, and 200 mg/litre and NAA at 0, 5, 10, and 20 mg/litre with an additional treatment hand thinned just after FB.
- Ethephon applied alone thinned effectively at 100 or 200 mg/litre.
- NAA applied alone did not thin at any concentration.
- The three concentrations of NAA with ethephon at 50 mg/litre improved thinning over the untreated control but not enough to significantly improve fruit weight and size.
- Ethephon alone at 100 mg/litre thinned very effectively and as well as any combination with NAA.
- Fruit weight and size were improved by all treatments using ethephon at 100 mg/litre.
- There was no advantage using ethephon at 200 mg/litre when compared to 100 mg/litre.
- Although there was some evidence that the combination of ethephon and NAA at the highest concentrations removed more fruit than ethephon alone at 200 mg/litre this was not reflected in better fruit weight or size.
- There is no advantage in adding NAA to ethephon to thin `Golden Delicious' at FB and ethephon at 100 mg/litre would be the thinning spray of choice.

Effects of fruit thinning agents on apple tree canopy photosynthesis and dark respiration

- Ralf Untiedt and Michael Blanke 2001
- Effects on photosynthesis of the fruit thinning agents naphthaleneaceticacid (NAA) and three commercial plant growth regulator formulations ,naphthaleneacetic acid ("Rhodofix") and naphthaleneacetamide("Amidthin") and 2-chloroethylphosphonic acid("Ethrel")were evaluated with respect to the stress they impose on the fruit tree, using the alternate-bearing sensitive apple cv. "Elstar".

- All employed thinning agents reduced whole tree canopy photosynthesis consistently by 3–34% on the five days following their application, with photosynthesis still declining thereafter in the case of the NAA and" Amid-thin" application.
- The reduction after application of either "Rhodofix" or "Ethrel", declined within five days, such that most of the original photosynthetic potential was restored, indicating acceptable phytotoxicity of these three plant growth regulators at the concentrations used.
- NAA and "Ethrel" *in*creased dark respiration over-proportionally by up to 106%, whereas "Amid-thin" and "Rhodofix" *de*creased it by up to 46% in the first night after application, thereby drastically affecting the carbon balance of the tree in opposite ways.

Citrus

Improving fruit size and packout of Late Valencia oranges with ethephon fruit-thinning sprays

Hutton 2005

- Ethephon (as Ethrel, 42-60 mL/100 L water), applied in a heavy-set year at 6-8 weeks post bloom when fruitlet size was 10-15 mm diameter, induced a 15-20% reduction in fruit number.
- This resulted in significantly improved fruit size and marketable outturn with negligible yield penalty.
- In the 2 successive harvests following spray treatment, the cropping pattern remained uniform and a 14% improvement in packout (marketable fruit size <100 counts per carton) was maintained.
- Internal fruit quality was unaffected.
- Control of alternate bearing was carried forward for at least 2 seasons with relatively stable yields following a single spray treatment.

Pear

Chemical thinning of Asian and European pear with ethephon and NAA

Steven and Rtney, 2002

- Asian pear (*Pyrus pyrifolia* Nakai) trees were sprayed with 400 ppm ethephon ((2-chloroethyl)phosphonic acid), applied 15 days after full bloom (DAFB) or hand thinned (leaving one fruitlet at each fruiting site 56 DAFB)
- Ethephon reduced fruit set of `Nijisseiki' and `Hosui' by 37 and 15%, respectively
- Fruit set was significantly reduced in proportion to the ethephon concentration with 800 ppm reducing fruit set by 62% compared to the nil treatment.
- Increasing ethephon concentrations significantly decreased mean fruit weight and flesh firmness at harvest, and significantly increased fruit soluble solids concentration and seed number.

Loquat

Chemical Fruit Thinning in Loquat with NAAm:Dosage, Timing, and Wetting Agent Effects

Cuevas *et al*., 2004

- Loquat is usually thinned by hand in February, but this practice is expensive and of limited usefulness due to its late execution.
- NAAm at doses of 30 and 60 mg l^{-1} applied at the end of bloom produced an average increase of fruit diameter of 11 and 18%, respectively.

Regulation of fruit drop, parthenocarpy and fruit development

Fruit drop

Causes

- The fruit lost are those they have not been completely pollinated.
- Competition between the fruits for food, water, and nutrients

Fruit drop and growth regulators

- The application of plant growth regulators can re-enforce hormone balance in the peel, reducing or retarding this precocious fall and the losses at harvest (Primo et al., 1966).
- Monselise and Goren (1978) reported that the spraying of auxins prevented the dropping of fruit by maintaining the cells at the zone of abscision, preventing the synthesis of hydrolitic enzymes, such as cellulase, which decomposed the cell walls.
- Citing Riov (1974), the same authors reported that hormone balance acted on the polygalacturonase activity, which, together with cellulase, was responsible for the degradation of the two important components of cell walls, cellulose and pectin.
- The use of 2,4-D as a growth regulator to promote size and to control fruit and leaf drop was reported by Hield et al. (1964)
- According to El-Otmani (1992), the combined application of GA_3 and 2,4-D reduces the precocious drop of fruit through the action of auxin and retards the softening and senescence of the peel, by the longer harvest time, and more economical storing in areas where stocking capacity is limited and cost is high.
- Prevention of pre-mature drop of fruits: 2,4,D,IAA,IBA, 2,4,5-T, are used to prevent pre-harvest deep of sweet oranges(100 to 500 ppm)

Control of premature fruit drop in Macadamia integrifolia: effects of naphthalene acetic acid application, cincturing, and shoot-tip removal.

Williams, 2003

- The effects of NAA application, cincturing and shoot-tip removal on the incidence of premature fruit
- A single, post-anthesis application of NAA (1 ppm) increased ($P < 0.05$) the final set of macadamia fruit by 35%.
- Limb cincturing combined with shoot-tip removal increased ($P < 0.05$) initial fruit set and possibly final set.

- Cincturing alone was less effective and shoot-tip removal had no effect alone.

Application of plant growth regulators at pre-harvest for fruit development of 'PÊRA' oranges

Almeida *et al*., 2004

- The treatments applied were: GA_3 + 2,4-D 12.5mg L^{-1} of each; GA_3 + 2,4-D 25mg L^{-1} ; GA_3 + 2,4-D 37.5mg L^{-1}; GA_3 + NAA 12.5mg L^{-1}; GA_3 + NAA 25mg L^{-1}; GA_3 + NAA 37.5mg L^{-1}; NAA + 2,4-D 12.5mg L^{-1}; NAA + 2,4-D 25mg L^{-1}; NAA + 2,4-D 37.5mg L^{-1}; and water (control).
- The treatments were applied 3 times, at intervals of 45 days. The variables evaluated were: rate of natural fall (%), fruit length and diameter (mm), and fresh fruit weight (g).
- None of the treatments promoted alterations in the development of the fruits, but they did reduce the natural fall rate, when compared to control, up to 78.05%, inhibiting the fruits' abscision as much as 3 months.

Effect of cobalt and silver ions and naphthaleneacetic acid on fruit retention in mango (*Mangifera indica* L.)

Alam *et al., 2004*

- The chemicals were applied to immature fruits at pea size (5-6 mm) and again 2 weeks later when the fruit lets had developed to marble size (10-15 mm).
- Three cultivars, Sindhri, Langra (3 seasons) and Dasehari (4 seasons), were tested and it was observed that all concentrations of the chemicals significantly ($P<0.05$) increased fruit retention.
- In Sindhri, cobalt nitrate, silver nitrate and NAA treatments increased yields by 106-165%, 89-146% and 94-115%, respectively, and in Langra by 100-135%, 98-138% and 95-118%.
- Whole tree sprays of cv. Dasehari with Co(NO3)2 at 100 mg/L, AgNO3 at 100 mg/L, and NAA at 20 mg/L yielded 129%, 66% and 54% more fruit than the control.

Hormonal control of fruit growth and fruit drop in mango cv dashehari

- The gibberellin content in seed increased rapidly during early seed growth and declined as growth decreased.
- The seed was the major source of gibberellin in the fruit, the pericarp containing only traces.
- Cytokinins were present both in pericarp and seed.
- During the single period of rapid growth in fruit and seed, cytokinin concentrations increased rapidly at two periods.
- The first rapid increase in cytokinin concentrations preceds the period of rapid cell division and cell enlargement and the second increase coincides with the period of rapid cell enlargement only.
- The level of ABA-like inhibitor was high in the first 21 d preceding pollination which corresponded with the period of slow growth in fruit and heavy fruit drop.
- During the rapid period of fruit growth, the level of inhibitors decreased and that of promoters increased.
- However, in maturation and slow fruit growth period, the levels of both the growth promoters and inhibitors were low.
- Thus all the growth promoters play their role in the growth of the fruit.
- Deficiency of auxins, gibberellins and cytokinins coupled with high level of inhibitors appear to cause fruit drop in mango cv. Dashehari.

Effect of aminoethoxyvinilglycine (AVG) on pre-harvest fruit drop and maturity of apples

- Apple trees cultivars Gala and Fuji were sprayed four weeks before commercial harvest with aminoethoxyvinilglycine (AVG), at doses of 0, 125, or 250 mg L^{-1}, and assessed for pre-harvest fruit drop, fruit growth, and maturation on tree.
- In 'Gala', 64 days after AVG spraying, fruit drop for control treatment was 85%, and AVG (at 125 and 250 mg L^{-1}) reduced it to 10%.

- In 'Fuji', 64 days after AVG spraying, fruit drop for control was 6%, while treatments with AVG (at 125 and 250 mg L^{-1}) increased fruit drop to 10%.
- AVG was a powerful retardant of fruit maturation for 'Gala' but not for 'Fuji'.
- In 'Gala', the most affected attribute was the skin background color, followed, in decreasing order, by soluble solids content, the starch index, skin red color, the flesh firmness, and titratable acidity. In 'Gala', only flesh firmness retention was improved by increasing AVG dose from 125 mg L^{-1} to 250 mg L^{-1}.
- The AVG at 250 mg L^{-1} inhibited "Gala" late fruit growth but not 'Fuji'.

Conclusions

- AVG substantially suppressed pre-harvest fruit drop and delayed fruit maturity on 'Gala' but not on 'Fuji';
- On 'Gala', the AVG dose of 125 mgL^{-1} might be used commercially to delay fruit harvest and increase yield by reducing premature fruit abscission and increasing fruit size of late harvested fruit;
- Fruit treated with AVG had poor skin color (dark-green background and deficient red color development) while still being able to mobilize starch, soften, and lose acidity on the tree.

Hormonal regulation of fruitlet abscission induced by carbohydrate shortage in citrus

- Sugar supply, hormonal responses and fruitlet abscission were manipulated through full, partial or selective leaf removals at anthesis and thereafter.
- In developing fruitlets, defoliations reduced soluble sugars (up to 98%), but did not induce nitrogen and water deficiencies.
- Defoliation-induced abscission was preceded by rises (up to 20-fold) in the levels of abscisic acid (ABA) and 1-aminocyclopropane-1-carboxylic acid (ACC) in fruitlets.

- Applications to defoliated plants showed that ABA increased ACC levels (2-fold) and accelerated fruitlet abscission, whereas norflurazon and 2-aminoethoxyvinyl glycine reduced ACC (up to 65%) and fruitlet abscission (up to 40%).
- Only the full defoliation treatment reduced endogenous gibberellin A_1 (4-fold), whereas exogenous gibberellins had no effect on abscission.
- The data indicate that fruitlet abscission induced by carbon shortage in citrus is regulated by ABA and ACC originating in the fruits, while gibberellins are apparently implicated in the maintenance of growth.
- In this system, ABA may act as a sensor of the intensity of the nutrient shortage that modulates the levels of ACC and ethylene, the activator of abscission.
- This proposal identifies ABA and ACC as components of the self-regulatory mechanism that adjusts fruit load to carbon supply

Amla

- Advances in flower and fruit regulation
- Three staged of flower and fruit drops
- I^{st} – highest as 70% of flower drop off with in three weeks of flowering due to degeneration of the egg apparatus and lack of pollination
- II^{nd} – from June - Sep – due to lack of pollination and fertilization
- III^{rd} – drop consist of fruit of various stages beginning from third week of Aug until Oct – due to embryological and physiological factors

Parthenocarpy

- In botany, the formation of fruits without seeds.
- This phenomenon, of no obvious benefit to the plant, occurs naturally in some plants, such as bananas.
- It can also be induced in some fruit crops, either by breeding or by applying certain plant hormones.

GA_3

- Gibberellic acid application (10 µmol pistil^{-1}) caused development similar to that in pollinated pistils, while benzyladenine (1 µmol pistil^{-1}) and naphthylacetic acid (10 µmol pistil^{-1}) treatment produced shorter siliques. Naphthylacetic acid primarily modified mesocarp cell expansion.

Auxins

- Parthenocarpy: IBA, NAA produces seed less/fruits - smaller sized fruits, but more in number, hence yield not affected.

Strong synergistic effects of gibberellins with the synthetic cytokinin N-(2-chloro-4-pyridyl)-N-phenylurea on parthenocarpic fruit set and some other fruit characteristics of apple

- The induction of parthenocarpic fruit set was investigated using the apple cvs. Golden Delicious and Jonagold.
- The gibberellins GA_3, GA_4, GA_5 and GA_7 and the synthetic phenylurea-type cytokinin CPPU (N-(2-chloro-4-pyridyl)-N-phenylurea), were applied alone and in combination to unpollinated flowers at the end of petal fall.
- Gibberellins induced only a marginal final set of parthenocarpic fruits.
- CPPU sprays were more effective, particularly in the first year.
- When applied in combination, CPPU and gibberellins had a positive synergistic effect on parthenocarpic fruit set and fruit size, but a negative effect on flower induction the next year.
- After CPPU + GA sprays, percent fruit set was similar, or greater, compared to natural pollinated trees.
- The parthenocarpic fruits induced by CPPU + GA had an increased length to diameter ratio.
- CPPU stimulated, and GA_4 and GA_7 reduced, the russeting of the parthenocarpic fruits.

- The internal quality of the fruits was hardly affected, but Ca-deficiency symptoms occurred more frequently in parthenocarpic fruits

Parthenocarpic fruit development in cloudberry (Rubus chamaemorus L.) is induced by 3b-hydroxylated gibberellins

- The purpose of this study was to test the activity of gibberellins and auxins for induction of parthenocarpic fruit development in cloudberry, Rubus chamaemorus L., a dioecious, northern wild berry
- Plant hormones were applied directly to open flowers, either dissolved in ethanol or as an aqueous spray.
- Of the tested gibberellins (GA_1, GA_3, GA_4, dimethyl-GA_4, GA_5, GA_9, GA_{19}, GA_{20} and GA_{24}) only GA_1, GA_3, GA_4 and dimethyl-GA_4 were active and induced fruit development comparable to development of pollinated fruits.
- Lack of activity of GA_{20} and GA_9, the immediate precursors of GA_1 and GA_4, respectively, suggests that 3b-hydroxylation of gibberellins can be inhibited in unpollinated flowers of cloudberry.
- Auxin treatments induced an initial fruit development, but did not result in ripe berries.
- Based on these results, use of transgenic methods to regulate gibberellin or/and auxin biosynthesis in carpels of cloudberry could be used to develop a parthenocarpic cloudberry.

Fruit set and development of three pear species induced by gibberellins

- Parthenocarpy was readily induced in two cultivars of Japanese pear when GA_{4+7} (500 ppm) in combination with or without other plant growth substances were applied on the emasculated and decapitated (stigma removed) flowers in full bloom and 3 weeks after.
- While GA_3 did not induce parthenocarpy in these cultivars.
- Gibberellin was not necessary for the second treatment (3 weeks after bloom), but could be replaced with NAA and/or BA.

- The parthenocarpic fruit was small and generally elongated-obovoid, different from the round seeded fruit.
- Parthenocarpic fruit set of European pear induced by GA_3, GA_4+$_7$ or GA_{45} was 42, 45 and 46%, respectively.

Fruit growth

Using urea phosphate to enhance the effect of gibberellin A_3 on grape size

- Gibberellic acid (GA_3) is widely used to enlarge the berries of seedless grapes (*Vitis vinifera* L). In cv. 'Sultana' (Thompson Seedless) the addition of 1000 mg/L urea phosphate (UP) to GA_3 solutions after fruit set reduced the pH of the solutions to a stable pH 2.9 and enhanced the effect of GA_3 on berry size and delayed maturation.
- Addition of citrate buffer, pH 2.9, to GA_3 sprays did not affect berry size or maturation.
- The possibility of improved GA penetration due to the low pH is considered.
- The nutritional effect of UP and direct enhanced penetration by the urea ion are also discussed.

Studies on apple fruit abscission and growth as affected by cytokinins

- Thinning trials were conducted for two years (1998-1999) on trees of Gala-derived cultivars ('Mondial Gala' and 'Galaxy'), using NAA and CPPU.
- In both cultivars CPPU, alone or in combination with NAA, has proven rather successful, always increasing fruit size.
- In both years and for both active ingredients the most effective concentration was 10 ppm, at various stages of fruit ontogeny.
- These applications resulted in reduced crop density (fruit number per trunk cross sectional area, TCSA) and always increased fruit size compared to hand-thinned controls.

- CPPU was also tested as a promoter of fruit growth, applied after trees had been thinned to optimum fruit load.
- Twenty ppm *a.i.,* applied when the king fruit reached 20 mm diameter, increased fruit size, improving yield per tree.
- Earlier modelling work on fruit growth has led to the hypothesis whether CPPU's mode of action might be via a cell division stimulation, resulting in greater numbers of cells in the cortical parenchyma of the treated fruits.

A Summary of growth regulator treatments used around the world.

Growth Regulator	Effect	Timing	Concentration	Extent of Effect
GA	Reduce flower number to increase fruit size.	Early June and at bud break.	One, (or for greater effect) two sprays of 25 ppm two weeks apart. Ralex® at label	Proportional to crop size, variety and tree vigour. 20% reduction in flowering in Australian trials. Registration of Ralex® due 2002,
GA	Improve fruit set.	70-90% petal fall (October).	10 to 25 ppm.	Proportional to crop size, variety and tree vigour.
Ethephon	Thin crop load	When fruitlets are 10 to 15mm in diameter	50-70ml/100Lat about 3500L/ha	Cost effective thinning agent. Good application techniques required.
3,5,6-TPA	Thin crop load especially smaller fruitlets.	When fruitlets are 15 to 17 mm in diameter	15ppm	Proportional to crop size, variety and tree vigour.
3,5,6-TPA	Expand cell size to increase fruit size.	When fruitlets are 20 to 30 mm in diameter	15ppm	Proportional to crop size, variety and tree vigour.
2,4,-D	Expand cell size to increase fruit size.	When fruitlets are 5 to 19 mm in diameter	57-110g/ha@ 5000L/ha.	Proportional to crop size, variety and tree vigour. Isopropyl ester formulation.
Dichlorprop (2,4,DP)	Expand cell size to increase fruit size.	Mandarins: 15 to 20 mm in diameter Oranges: 22 to 25 mm in diameter	50 to 100 ppm.	Proportional to crop size, variety and tree vigour. A 4 to 5 mm increase in fruit diameter for mandarins and a 5 to

NAA	Crop thinning	When fruitlets are 15 to 20 mm in diameter	200-350ppm	Temperature dependant (rates vary to ambient temperatures)

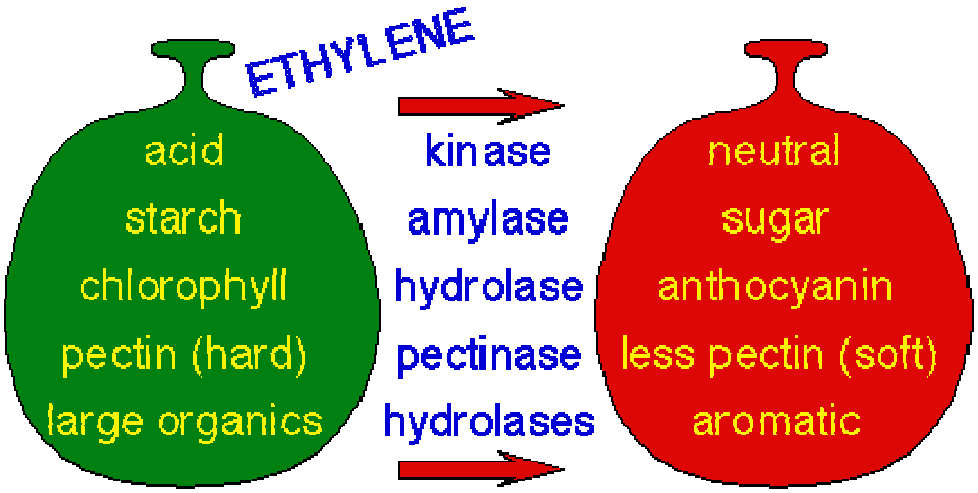
Summary of Sprays Trialed in California (240 trees/ha)

Nutrient	Rate	Timing	Effect
Urea (low biuret)	0.5%Nat31.4kgN/ha (1.1 kg/100L low biuret urea @ application volume 6300L/ha)	Winter- Pre bloom	Increase yield without reducing fruit size. Also reported to increase TSS at harvest.
Phosphorus (Phosphite)	Nutri-phite® 0-28-26 [phosphorus & potassium mixture] at 7.3L/ha	Winter- Pre bloom	Increase yield without reducing fruit size. Also reported to increase TSS at harvest.
Urea (low biuret)	1.3%Nat31.4kgN/ha (2.8kg/100L low biuret urea @ application volume 2400L/ha)	Full bloom	Increase yield by increasing fruit set. Fruit size is not reduced.
Urea (low biuret)	1.5%Nrateat31.4kgN/ha (3.3kg/100L low biuret urea @ application volume 2100L/ha)	Maximum Peel Thickness / End of cell division (Early/Mid Summer)*	Increase fruit size
Phosphorus (Phosphite)	Two applications of Nutri-phite® at 4.6L/ha	Mid/late spring and early/mid summer*	Increase fruit size and increase TSS ratio at harvest.

Ex.No.9. Assessment of physiological changes during fruit ripening

Regulation of ripening and storage

Aspects include fruit ripening and maturation, postharvest treatments to advance or retard ripening, control of storage decay, storage physiology, and effects on shelf-life, including vase life of cut flowers.



- An increase in expression of ACC synthase causes an increase in ethylene
- Ethylene accelerates the process of fruit ripening, which includes cell wall breakdown, production of pigments, can include a burst of respiration (climacteric) and sweetening
- Applied ethylene (as ethephon) can promote ripening of fruit before or after harvest
- Control of ethylene is important in post-harvest storage of fruit and flowers; can use low O_2, low temp, ethylene scavengers (also controls respiration)
- Can repress the expression of ACC synthase gene in tomato fruit and get delayed ripening

The role of ethylene in fruit ripening

- Ethylene is a plant hormone regulating fruit ripening by coordinating the expression of genes that are responsible for a variety of processes, including a rise

in respiration, autocatalytic ethylene production and changes in color, texture, aroma and flavor.

- Ethylene is biosynthesized from S-adenosylmethione via 1-aminocyclopropane-1-carboxylic acid (ACC), catalyzed by ACC synthase and ACC oxidase.
- Both enzymes are limiting in preclimacteric fruits but are greatly induced during the ripening.
- ACC synthase has been purified and characterized from various fruit tissues and its cDNAs cloned.
- ACC oxidase was identified by a reverse genetic approach and subsequent identification of gene function by expression in heterologous systems.
- ACC synthase and ACC oxidase are encoded by multigene families.

Banana

- Banana is commercially ripened by treatment with exogenous ethylene gas at 100 ppm maintaining pulp temperature between 16-18^{o}C at 95% RH

(Golding *et al*., 1998)

- Stored at a temperature above 13^{o}C and with a RH of 85-95% for about 3 weeks and is ripened in a week or two at 62^{o} – 70^{o} F (16.5 – 21^{o}C)
- Fruit becomes blackened at lower temperature and should not be placed in a refrigerator

Regulation of 1-MCP-Treated Banana Fruit Quality by Exogenous Ethylene and Temperature

Jiang *et al*., 2004

- Reductions in firmness, titratable acidity (TA) and starch content (SC) of banana fruits were remarkably delayed by 1-MCP treatment.
- 1-MCP treatment also delayed the increase of total soluble sugar (TSS) and soluble pectin (SP) contents.

- Soluble solids (SS) content in the 1-MCP-treated fruit remained almost unchanged during the first 10 days of storage at 20 C.

Ripening behaviour and quality of Brazilian bananas following hot water immersion to disinfest surface insects

Marisa, 2004

- Fruits exposed to 51ºC hot water for 20 min. increased the shelf life and keeping quality
- Hot water did not affect the total sugar or sucrose concentration of ripening banana
- Starch concentration remained higher than control.
- Glucose and fructose concentrations were reduced in banana immersd in 51ºC hot water for 20 min.
- Delayed respiratory peaks and ripening
- Decreased Co_2 production also tended to have the highest starch and lowest sugar concentration.
- Exposure to hot water for 20 min may have inhibited starch hydrolytic enzymes.
- Therefore less carbon was available to enter the glycolytic and gluconeogenic pathways.
- Ethylene production was reduced and delayed.
- Inhibit ethylene production in fruits through inactivation of 1-aminocyclopropane -1-carboxylic acid (ACC) synthase and ACC oxidase.

Effects of Chilling Temperatures on Ethylene Binding by Banana Fruit

Yueming Jiang *et al*., 2004

- Exposure of banana fruit to the ethylene binding inhibitor 1-methylcyclopropene (1 $\mu l\ l^{-1}$ 1-MCP) prevented ripening. However, this treatment also enhanced the chilling injury accelerated the occurrence of chilling injury-associated increased membrane permeability.

- ^{14}C-ethylene release assay showed that ethylene binding by banana fruit stored at low temperature decreased with reduced storage temperature and/or prolonged storage time.
- Fruit exposed to 1-MCP for 12 h and then stored at 3 or 8 °C exhibited lower ethylene binding than those stored at 13 °C.
- Thus, chilling injury of banana fruit stored at low temperature is associated with a decrease in ethylene binding. The ability of tissue to respond to ethylene is evidently reduced, thereby resulting in failure to ripen.

Grapes

Storage of 'Italia' grape submitted at calcium application.

Lima *et al*., 2000

- Calcium was applied by immersing the bunches in 0 and 1.5% $CaCl_2$ solution for 10 seconds at the initial phase of colour change and softening of berries.
- Grapes were stored under refrigerated conditions (3.3-3.6^{o}C and 87-99% relative humidity) and evaluated at 14-day intervals until day 70.
- Treatment with calcium decreased TSS, pH, and TSS/TAA ratio, and increased the TTA and calcium content in the rachis and berry.
- The storage life of the grapes was =56 days.

Use of different wax films for the prevention of dehydration of the rachis of table grapes post harvest.

Baez Sanudo *et al*., 2001

- Edible films or coatings could be a good alternative to prevent the problem since this compound modulates the tissue permeability, decreasing water loss and dehydration rate.

Papaya

Ramakrishna and Hari Babu. 2003. **Effect of post harvest application of $CaCl_2$ and Wax emulsion on the storage life of papaya**

- $CaCl_2$ 3% and wax emulsion 6% - reduced PLW
- Wax coating reduced water loss – controlled transpiration
- $CaCl_2$ – maintain cellular organization and regulating enzyme activities and thereby help in reducing water loss.
- TSS maximum in control (11.01%), less in $CaCl_2$ 3.0% and 6% wax at 12 days of storage.
- Increase in TSS – conversion of starch and other polysaccharides into sugars.
- $CaCl_2$ (3.0%) and wax emulsion (6.0%) – high titrable acidity (0.31%) after 12 days of harvest
- Changes in acidity – changes in mechanisms of respiratory process and conversion of acids to sugars.
- Sugar content was maximum in control (8.92%), low total sugar (7.67%) – $CaCl_2$ 3% followed by 6% wax.
- Carotenoid maximum in control (1.52%) compared to 3% $CaCl_2$ and 6% wax – slowed process of conversion of chlorophyll to orange coloured pigments.
- Shelf life → 6% wax (10.67), $CaCl_2$ 3% (10.33); control (6.33)
- Post-harvest treatments – extended post harvest life by another 4 days.

Guava

Effect of postharvest application of ethephon and calcium carbide on the ripening behaviour and quality of Guava fruits

Mahajan *et al.*, 2004

- Ethephon 500 ppm recorded minimum weight loss
- The Ethephon 500ppm treated fruits were found quite firm in texture due to lower water loss from the fruits as a result of regulation of ripening by ethylene action
- 1000ppm Ethephon registered lowest spoilage % after 4 days due to reduction in primary inoculam load in the fruit by aqueous dip

- 1000ppm attained uniform ripening and color due to controlled ripening mechanism initiated by ethylene action
- Increase in TSS and ascorbic acid in fruits treated with ethephon 1000ppm was more than Ca carbide

Apples and pears

Effects of some growth regulators on the ripening and storage quality of apples and pears

Sharples *et al*., 1986

- Aminoethoxyvinylglycine (AVG) appears to inhibit ripening by blocking ACC synthase activity and its effects can be overcome by treatment with exogenous ethylene.
- Daminozide also delays the rate of ripening by suppression of ethylene synthesis and, where pre-harvest spray application of daminozide is supplemented by efficient ethylene removal, significant improvements in the quality of apples stored in controlled atmospheres may be achieved.
- The increase in core flush (brown core) and breakdown which may occur in some varieties after treatment with daminozide can be largely overcome by reducing application rates and selecting appropriate storage conditions.
- Cycocel (CCC) appears to have relatively little effect on fruit composition or ripening rate but Paclobutrazol promotes fruit calcium levels and, in some trials, has improved fruit firmness and increased acidity.
- These effects are consistent with those of an anti-gibberellin treatment since GA sprays applied close to full bloom, can depress fruit calcium levels and lead to an increase in disorders during storage.
- Tri-iodobenzoic acid has also been shown to depress fruit calcium levels and to lead, in some cases, to increased bitter pit.
- Synthetic auxin sprays applied to prevent premature shedding before harvest time increase fruit red colour and may advance ripening in certain varieties.

- Post-havest application of GA and IAA may have beneficial effects on the storage qualities of several varieties of apples and pears.
- Injection of GA into the core of Jonathan apples reduces breakdown whereas abscisic acid tends to promote this disorder.
- It is evident that application of growth regulators to harvested fruits may often produce very different effects in the stored crop to those observed when the same compound is applied to the fruiting tree.

Effects of some growth regulators on the development and ripening of peach fruits

Gyuró and Dávid 1978

- On peach cv. Marygold, spraying with 5000 ppm SADH at the stage of rapid initial growth encouraged earlier and more uniform fruit ripening.
- The most effective results followed an application of an SADH-Ethrel mixture sprayed at stone-hardening.
- This gave only 6.5% unripe fruit at harvesting compared with 11% in the control. Succinic acid at 2000 ppm showed results similar to those of SADH.

Ex.No.10. Estimation of ascorbic acid content in fruits

Ascorbic Acid

Volumetric Method

Ascorbic acid is otherwise known as Vitamin C and is an antioxidant. It is present in goose beery, bitter gourd etc. in high amounts. Generally it is present in all fresh vegetable and fruits. It is a water soluble and heat-labile vitamin. The method described below is easy, rapid and a large number of samples can be analysed in a short time.

Principle

Ascorbic acid reduce the 2, 6-dichloro phenol indophenols dye to a colourless leuco-base. The ascorbic acid gets oxidized to dehydro ascorbic acid though the dye is a blue coloured compound, the end point is the appearance of pink colour. The dye is pink coloured in acid medium.

Materials

- Oxalic Acid 4%
- Dye solution – Weight 42 mg sodium bicarbonate into a small volume of distilled water. Dissolve 52 mg 2, 6-dichloro phenol indophenol in it and make up to 200 ml with distilled water.
- Stock standard solution – Dissolve 100 mg ascorbic acid in 100 ml of 4% oxalic acid solution in a standard flask (1 mg/ml).
- Working standard – Dilute 10 ml of the stock solution to 100 ml with 4% oxalic acid. The concentration of working standard is 100 mg/ml.

Procedure

1. Pipette out 5ml of the working standard solution into a 100 ml conical flask.
2. Add 10ml of 4% oxalic acid and titrate against the dye (V_1ml). End point is the appearance of pink colour which persists for a few minutes. The amount of the dye consumed is equivalent to the amount of ascorbic acid.
3. Extract the sample (0.5-5 g) depending on the sample in 4% oxalic acid and make up to a known volume (100 ml). Centrifuge or filter.

4. Pipette out 5 ml of the supernatant, add 10 ml of 4% oxalic acid and filtrate against the dye (V_2 ml).

Calculation

$$\text{Amount of ascorbic acid mg/100 g of sample} = \frac{0.5\text{mg} \times 2 \times 100\text{ml}}{V_1\text{ml} \quad 5\text{ml}} \times \frac{100}{\text{Wt of sample}}$$

Result

The amount of ascorbic acid present in the given sample = ----------mg.

Ex.No.11. Estimation of TSS and total sugar content

TSS

The total soluble solids of each sample was estimated through a hand refractometer and expressed as degree brix.

Estimation of total sugar content

1. Total sugar content

Procedure

Reagents used were 2.5 N HCl, anthrone reagent, standard glucose and working standard (10 ml standard glucose diluted to 100 ml with distilled water).

One hundred mg of the sample was taken in a boiling tube and the sample was hydrolyzed by keeping it in boiling water bath for 3 hours with 5 ml of 2.5 N HCl. The whole content was cooled to room temperature. It was neutralized with sodium carbonate until the effervescence ceased. Then the volume was made up to 100 ml with distilled water and the supernatant was collected after centrifuging. Aliquots of 0.5 and 1.0 ml were taken for analysis.

The standards were prepared by taking 0.2, 0.4, 0.6, 0.8 and 1.0 ml of the working standard. Then, the volume was made up to 1.0 ml with distilled water and 4 ml of Anthrone reagent was added to all the test tubes and heated for 8 minutes in boiling water bath. The tubes were then cooled rapidly. The OD of the green coloured product was read at 630 nm. The standard graph was drawn by plotting concentration of the standards on the x-axis and absorbance on the y-axis and, the amount of total sugars present was calculated by using the following formula.

$$\text{Amount of total sugars present in 100 mg of the sample} = \frac{\text{mg of glucose}}{\text{Vol. of test sample}} \times 100$$

Ex.No.12. Estimation of carotenoids and lycopene content in fruits

Carotenoids

The Carotenoids are a group of yellow, orange and orange red fat – soluble pigment widely distributes in nature. In green leaves, they occur in grana of chloroplast. The green colour of the chlorophyll masks the yellow to red colour of the carotene except in very young leaves and in variegated leaves, wherein chloroplast content is less. These pigments are present in mango, papaya, tomato, carrot, red pepper, most of the flowers, etc. Carotenoids are of two groups carotenes and xanthophylls. Carotenes are hydrocarbons with the empirical formula $C_{40}H_{56}$ composed of eight isoprene units. There are several isomers of which β- carotene, the precursor of vitamin A, is most abundant and may make up as much as 0.1% of the leaf dry weight. The red pigment, lycopene, found in ripe tomatoes, rose fruits and other plant parts, is also as isomer of carotene.

Most xanthophylls have the formula $C_{40}H_{56}O_2$, and are yellow to brown in colour. Xanthophylls can be separated physically from carotene because they are more soluble in alcohol and much less soluble in petroleum ether. The most abundant leaf xanthophyll is lutein which sometimes occurs in higher concentration that β- carotene. Carotenoids function as accessory pigments in photosynthesis and may also protect chlorophyll form irreversible photo oxidation.

Estimation of carotenoids

Procedure

250 mg of fresh leaf sample is macerated thoroughly with 80% acetone (10ml) using a pestle and mortar. The leaf extract is centrifuged at 4000 rpm for 20mts and the residue is re-extracted with another 5ml of 80% acetone until homogenate becomes colorless. The supernatant solution is transferred to a 25ml volumetric flask and made up to 20ml with 80% acetone. The optical density of the extract is measured at 480 and 510nm wavelength in a Spectrophotometer.

Calculation

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

The carotenoid content is expressed as **mg g-^{1}** of fresh leaf.

Estimation of lycopene

Procedure

Ten grams of sample was extracted with acetone. The acetone extract was transferred to a separating funnel containing 15 ml of petroleum ether and mixed gently. The lower acetone phase was diluted with water containing five per cent sodium sulphate and then transferred to another funnel. The extraction was repeated with petroleum ether until it was colourless. Anhydrous sodium sulphate was added to the pooled petroleum ether extracts and the volume was made up to 100 ml with petroleum ether. An aliquot of 5 ml was diluted to 50 ml and the colour was read at 503 nm in a spectrophotometer against petroleum ether as blank. The lycopene content of the sample was expressed as mg/ 100g and calculated by the formula.

Calculation: $\frac{3.1206 \times \text{OD value of sample} \times \text{Volume made up} \times \text{Dilution}}{1.0 \times \text{weight of sample} \times 1000} \times 100$

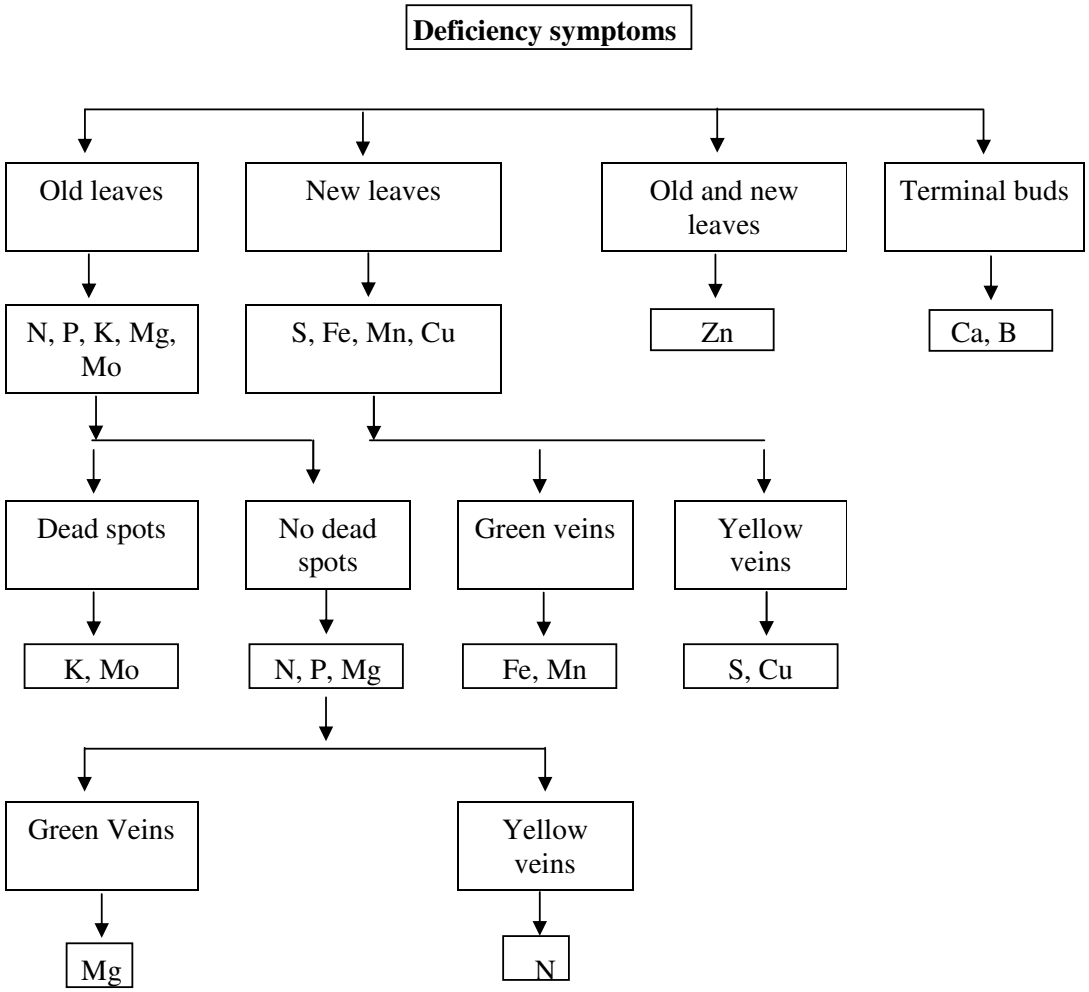
Ex.No.13. Identification of physiological and nutritional disorder and correction measures in horticultural crops

Deficiency Symptoms

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.



Identification of deficiency symptoms

The region of appearance of deficiency symptoms depends on mobility of nutrient in plants. The nutrient deficiency symptoms of N, P, K, Mg and Mo appear in lower leaves because of their mobility inside the plants. These nutrients move from lower leaves to growing leaves thus causing deficiency symptoms in lower leaves.

Zinc is moderately mobile in plants and deficiency symptoms, therefore, appear in middle leaves. The deficiency symptoms of less mobile elements (S, Fe, Mn and Cu) appear on new leaves. Since Ca and B are immobile in plants, deficiency symptoms appear on terminal buds. Chlorine deficiency is less common in crops.

Deficiency symptoms on old leaves

The symptoms that appear on old leaves can be further distinguished based on the presence or absence of dead spots.

Without Dead Spots. The characteristic deficiency symptom of nitrogen is uniform yellowing of the leaves including the veins. The leaves become stiff and erect especially in cereals. The leaf may detach after a little forceful pull in extreme deficiency in dicotyledonous crops. Cereal crops show characteristic 'V' shaped yellowing at the tip of lower leaves.

In phosphorus deficiency, leaves are small, erect, unusually dark green with a greenish red, greenish brown or purplish tinge. The rear side develops bronzy appearance.

Magnesium deficiency also causes yellowing, but differs from that of nitrogen. The yellowing takes place in between the veins and the veins remain green. The leaf is not erect. The leaf detaches very easily and may be shed by blowing wind. Necrosis (death of tissues) occurs in extreme cases only in the margins.

With Dead Spots. In potassium deficiency, yellowing starts from tips or margins of leaves extending to the center of leaf base. These yellow parts become necrotic (dead spots) very soon. There is sharp difference between green and yellow and yellow and necrotic portions. The dead spots appear particularly on margins and tips.

Molybdenum deficiency causes translucent spots of irregular shape in between the veins of leaves. These spots are light green, yellow or brown in colour. The affected spots are impregnated with resinous gum which exudes from rear side of the leaf from the reddish brown spots.

Deficiency Symptoms on New Leaves

These symptoms may be spread over entire leaf or the veins may remain green.

Veins Remaining Green. Veins remain green in iron and manganese deficiency. In iron deficiency, the principal veins remain conspicuously green and other portions of the leaf turn, yellow tending towards whiteness. Under severe deficiency, most part of the leaf becomes white. In manganese deficiency, the principal veins as well as the smaller

veins are green. The interveinal portion is yellowish, not tending towards whiteness. Dead spots also appear at a later stage. There is a chequered appearance to the leaf.

Veins not Remaining Green. The leaf becomes yellowish due to sulphur deficiency, but looks like nitrogen deficient leaf. The leaf is small and the veins are paler than intervenial portion. No dead spots appear. Plant does not lose the lower leaves as in the case of N deficiency.

In copper deficiency, leaf is yellowish tending towards whiteness. In extreme deficiency, chlorosis of veins occur and leaf loses luster. Leaf is unable to retain its turgidity and hence, wilting occurs. Leaf detaches due to water soaked conditions of the base of petiole.

Terminal Buds

The deficiency symptoms of Ca and B are many times seen on new leaves. However, it is easy to recognize their deficiency symptoms on the terminal buds or growing points than on new leaves.

In calcium deficiency, the bud leaf becomes chlorotic white with the base remaining green. About one-third chlorotic portion of the tip hooks downward and becomes brittle. Death of terminal bud occurs in extreme cases.

Boron deficiency causes yellowing or chlorosis which starts from the base to tip. The tip becomes very much elongated into a whip like structure and becomes brownish or blackish brown. Death of the terminal bud occurs in extreme cases.

Deficiency on both old and new leaves

In zinc deficiency, the leaf becomes narrow and small. Lamina becomes chlorotic and veins remain green. Subsequently, dead spots develop all over the leaf including veins, tips and margins. In cereals, zinc deficiency generally appears in 2-4 leaves from the top during vegetative stage. Plants appear bushy due to reduced internodal elongation. Subsequently, panicle fails to emerge completely or emerges partially.

Toxicity Symptoms

When a nutrient is present in the soil in excess of plant's requirement, the nutrient is absorbed in higher amounts which causes imbalance of nutrients or disorder in physiological processes. Unlike deficiency symptoms, toxicity symptoms are less common.

Nitrogen: Excess nitrogen causes delay in maturity and increases succulency. The adverse effects of excess nitrogen are lodging and abortion of flowers. Crop becomes susceptible to pests and diseases.

Phosphorus: Excess phorphorus causes deficiency of iron and zinc. In some crops like maize, leaves develop purple colouration and plant growth is stunted. In cotton, leaves become dark green in colour, maturity of bolls delayed and stems turn red.

Iron: Tiny brown spots appear on the lower leaves of rice starting from tips and spreading towards bases. Leaves usually remain green. In extreme case, the entire leaf turns purplish brown in colour.

Manganese: The plant is stunted and tillering is often limited. Brown spots develop on the veins of the leaf blade and leaf sheath, especially on lower leaves. Manganese toxicity occurs in lowland rice.

Boron: Chlorosis occurs at the tips of the older leaves, especially along the margins. Large, dark brown, elliptical spots appear subsequently. The leaves ultimately turn brown and dry up.

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 are, bronzing (development of bronze or copper colour on the tissue), chlorosis (loss of chlorophyll resulting in loss of green colour leading to pale yellow tissues), decline (onset of general weakness as indicated by loss of vigour, poor growth and low productivity), die-back (collapse of the growing tip affecting the younger leaves), firing (burning of tissue accompanied with dark brown or reddish brown colour), lesion (a localized wound of the leaf/stem tissue accompanied with loss of normal colour), necrosis (death of tissue), scorching (burning of the tissue accompanied with light brown colour resulting from faculty spray, salt injury, etc.)

Some crops are more sensitive than others to the deficiency of a micronutrient and it can be inferred that the critical concentration of a nutrient is not same for all the crops. The susceptibility or tolerance rating of crops to nutrient deficiencies shows considerable variation due to wider hereditary variability within a crop species (Tandon, 1995).

Apart from the nutrient deficiencies that affect various horticultural crops, physiological disorders are also commonly noticed. 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.

For the proper growth and development of crop plants, especially fruit trees, major nutrients such as nitrogen, phosphorus and potassium, secondary nutrients such as magnesium, calcium, sulphur and micronutrients such as iron, magnesium, copper, zinc, boron and molybdenum are very much needed in proper quantities. If even any one of the aforesaid nutrient elements is available is less than optimum level in the plant, the very growth of the plant is adversely affected. Hence, it is imperative that all the essential

nutrient elements should be adequately provided to the crop plants so as to facilitate the tissues in carrying out the various metabolic and physiological processes.

When foliar spray of fertilizer is taken up, it is advisable to have the spray either in the morning or evening. While spraying, the foliage should be drenched fully and it can be achieved by operating a hand sprayer. Addition of soap solution to the spray solution is very much required to facilitate uniform spread and wetting of the nutrient solution on the leaf surface. Rainy days should be avoided for the foliar spray. When soil application of nutrient mixtures is adopted, the fertilizers should be mixed with compost farm yard manure which helps in easy availability and uptake by the plants.

Banana

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.

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

It is the dislodgement of individual banana fingers from the bunch during ripening and markting. It can be defined as physiological softening and weakening of the pedicel which cause the individual fruit of a hand to separate very easily from the crown. 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. Softening of fruit is associated with the water relations of cell and composition of cell walls. 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. Kottaivazhai

It is a serious malady in Poovan variety of banana, reducing the production by 10-25%. The symptoms are distinctly conical and ill filled fruits with a prominent central core having many underdeveloped seedy structures making the fruit inedible. The pseudostem exhibits streaks, striations and blotches on the surface. Bunches are held at an angle above the horizontal position. Pollen grains are infertile, shriveled, shrunken and broken while the pericarp is smaller and the locular cavity is bigger than normal. The

absence or the occurrence of auxin, gibberellin and cell dividing factors at sub epidermal levels affect the development of parthenocarpic fruits. Application of 2, 4-D 25 ppm and GA 100 ppm after the opening of last had favours development of parthenocarpic fruit.

6. 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. Occurrence of hard lump is seasonal and only the bunches shot during dry winter period exhibit the phenomenon. 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.

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.

Nitrogen deficiency significantly delays plant growth and development by reducing leaf production rates as well as the relative distance between leaves. A rosette appearance often results from each leaf emerging and developing on top of each other. Plant height and leaf lengths are also considerably reduced.

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. Deformations of the flag leaf can be induced by poorly placed herbicide applications such as glyphosate and can be erroneously confused with deficiencies of Ca, Zn or B. Similarly, symptoms of viral attack can be mistaken for deficiencies of these elements.

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. Another Mg deficiency symptom is a bluish-purple colouration of the petioles called blue sickness.

When Mg deficiency is severe, the basal section of the petiole can separate and break from the pseudostem, causing early leaf senescence.

5. Zinc deficiency

Due to imbalanced fertilizer application and high density planting, incidence of Zn deficiency has become yield limiting. Disproportionate and high application of DAP as basal and top dressing create P induced Zn deficiency in banana. The leaf width is 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.

2. Mango

Physiological disorders

1. 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. The preventive measure is to have orchards 1.5 km to the east and west and 0.75 kg to the north and south away from the kilns. Spraying of 2% sodium carbonate or 0.6% borax is recommended as control measure.

2. Scorching of leaves

It has become a serious problem in north India. The typical symptoms are the brick red colour towards the tip, along the margins of old leaves and subsequent collapsing of these tissues. New leaves do not exhibit any such deformities. Almost all leaves get affected in the winter. The symptom resembles potassium deficiency, but Pandey (1971) reported that it is caused by chloride ion toxicity. For K application, the use of potassium chloride should be avoided, instead potassium sulphate should be used. Irrigation water having high chloride content should not be used.

“Deficiency symptoms”

The leaves will become smaller in size, interveinal chlorosis will set in which may lead to reduction in photosynthesis. As a consequence, fruit yield will be very much affected.

Recommendations

The aforesaid symptoms are manifested as a result of deficiency of magnesium and zinc. Hence, it is advisable to provide the mango tree with the following nutrients: 250 g. each of magnesium sulphate, manganese sulphate, zinc sulphate and borax are mixed along with 100 gm of urea in 100 litres of water added with 100 ml of soap solution. The solution is given through foliar spray three times at 20 days interval. It is also followed up with soil application at the rate of 75 gm each per tree of magnesium sulphate, zinc sulphate, manganese sulphate and borax along with 20-25 kg of compost, irrigation should be done immediately after soil fertilization.

Acid lime

“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. Besides, soil application of manganese sulphate (100 gm) and zinc sulphate (100 g) along with 25 kg of compost farm yard manure will be advantageous.

Acid lime and jack

1. "Zinc Deficiency"

It is characterized by small leaves with shorter stalks producing a rosette appearance. Interveinal chlorosis will be noticed. The fruits will be smaller in size and the yield will get affected because of higher proportion of fruit drops.

Recommendations

Foliar application of nutrient solutions contains zinc sulphate (500 gm) and lime (250 gm) mixed with 100 gm of urea and 100 ml soap solution dissolved in 100 litres of water is given thrice at 20 days intervals. When the tree put forths new growth, basal soil application of 250 g zinc sulphate along with compost FYM during March and September in equal doses is most beneficial.

Sapota

1. "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

Symptoms

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

Since this malady is brought about by a deficiency of a combination of nutrients, the following micro nutrients will be applied through coliar spray. 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

1. "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.

2. "Zinc deficiency"

Zinc deficiency is sometimes implicated as a cause for this disorder. Hence, it is preferable to mix zinc sulphate @ 500 g with borax and urea (100 g) dissolved in 100 litres of water and sprayed through foliar means.

Soil application of borax (75g) and zinc sulphate (75g) along with 25-30 kg of FYM per tree is also adopted.

Tomato

1. "Blossom End Rot"

This deficiency disorder is caused by insufficiency of calcium in the soil, usually induced by water stress in the plant. At time of fruit set, cells at the blossom end of fruits are injured when insufficient calcium translocation to the flower results in a dry rot brown area on the expanding fruit.

Recommendations

Soil moisture should be maintained at optimum level so as to prevent the occurrence of this malady. To correct this disorder, foliar spray of lime (calcium chloride) @ 500 g dissolved in 100 lit of water along with 100 ml of soap solution for one acre of garden. While spraying, it should be ensured that complete drenching of the fruits with the spray solution is achieved.

Apple

1. "Internal Cork"

The mesocarp of the fruit will become corky with dark brownish patches thus rendering it unfit for consumption. It is due to born deficiency.

Recommendations

Foliar application of borax (500 g) and urea (100 g) dissolved in 100 lit of water will remedy the disorder.

Nutritional disorders and corrections in vegetable crops

Vegetables are very often subjected to nutrient stresses because of their rapid growth, high nutrient requirement and intensity of production. The requirement of nutrients for vegetable crops is considerably higher than that of field crops. It has been observed that nitrogen should be high in fertilizers for leafy foliage crops, that phosphorus should be high for fruit crops and potash high for root crops.

Symptoms of nutrients deficiencies that develop in vegetable crops grown in the field often vary from those produced in plants grown under controlled greenhouse

conditions. Disease and insects often produce plant symptoms closely resembling those resulting from nutrient deficiencies.

Cauliflower

"Brown heart disorder"

Boron deficiency in cauliflower, turnip, radish, cauliflower and other root crops, commonly cause what is known as brown heart. It is manifested first by dark spots on the roots, usually on the thickest parts. The plant gradually becomes stunted or dwarfened. The leaves are smaller than normal and fewer.

In the case of cauliflower, the flower head will show hollow stem and bronzing due to the decay of the core tissues of the stalk.

Recommendations

To ameliorate this disorder, foliar spray of 500 g of borax along with 100 g of urea and 100 ml of soap solution dissolved in 100 lit of water has to be taken up in an acre of land. Spray is to be repeated two to three times at an interval of 20 days.

Chillies

"Little leaf with intervenial chlorosis"

Symptoms

The chillies crop has high affinity for zinc nutrition and hence large amount of zinc is taken up by the plant. If zinc is not replenished periodically, the crop is prone to suffer from zinc deficiency severely.

The entire foliage of the crop will be reduced in size with interveinal chlorosis, when zinc is in short supply and the plant will present a stunted growth.

Recommendations

Foliar application of zinc sulphate @ 500 g and urea @ 100 g dissolved in 100 lit of water along with 100 ml soap solution is to be given two to three times at an interval of 20 days depending upon the extent of severity.

Foliar application has to be supplemented with soil application of zinc sulphate @ 8 kg/acre is given basally during last ploughing of the field.

Brinjal

"Iron Chlorosis"

Symptoms

Young as well as older leaves will exhibit chlorotic symptoms in the form of yellwing. So there will be a decline in photosynthesis due to which fruit yield will be greatly reduced.

Recommendations

Foliar spray of solution containing 500g of ferrous sulphate and 100 g of urea dissolved in 100 lit of water added with 100 ml of soap solution for an acre of land is taken up two or three times at an interval of 20 days depending upon the degree of severity.

Coconut

"Pencil point disease"

This is a physiological disorder affecting coconut palms to a great extent in areas such as Tanjore, Thiruvarur, Pattukottai, etc. The onset of this disorder is brought about by blockage of vascular bundles which may lead to disruption in the transpiration process.

The typical symptom is characterized by the tapering of truck towards the crown, the fronds will become smaller, rigid and erect. The outer fronds will first show yellowing and drop off. As a result, the number of fronds will be reduced. The nuts will be fewer in number and smaller in size; most of the nuts will be hollow. Thus the yield of nuts is severally affected once this disorder sets in the coconut palm.

Recommendations

Along with the recommended fertilizer dose, 225 g each borax, zinc sulphate, manganese sulphate, ferrous sulphate, copper sulphate and 10 g of ammonium molybdate

may be dissolved in 10 litres of water and poured in the basin of 1.5 m radius from the trunk of the palm.

Flower crops

Jasmine

"Nematode induced chlorosis"

Symptoms

The foliage will exhibit interveinal chlorosis as a result of iron deficiency in the soil, when the plant is infested with nematode the iron and other nutrient availability by translocation to the plant tissues is hampered due to blockage of vascular bundles as a result it is made to suffer from shortage of iron and other nutrients as also water. The leaves will have fade yellow colouration; the growth of the plant is stunted which may eventually lead to the wilting of the entire plant. The flower yield will be substantially reduced.

Amelioration

The iron chlorosis is a common occurrence in jasmine as many of the areas where it is widely grown has been infested with nematode. So, control of nematode infestation has to be taken up on priority basis.

After digging the soil around the tree, the following are applied to the soil: 10 g of Temic or 20 g of Furadon, 250 g of Neem cake and 50 g of murate of potash. It is followed by irrigation and application of 0.1% emison solution. After one week, foliar application of 500 g of ferrous sulphate and 100g of urea along with 100 ml of soap solution mixed in 100 lit of water is done two to three times at 20 days interval depending upon the extent of severity.

Rose

Failure of bud to blossom is a serious physiological disorder in rose which affects the marketability of the rose flower.

Recommendations

The fungicide Bavistin is taken @ 100 g and dissolved in water and the clear solution is further used for dissolving 1 kg super phosphate along with 100 g urea and 100 ml of soap solution. The entire mixture is added to water the total volume of which is made up to 100 litres. This volume of water is sufficient for spray in an area of one acre of land.

Crossandra

"Nematode Induced Chlorosis"

Symptoms

Crossandra is also affected by nematode induced chlorosis just like jasmine. In this crop, the typical symptom is characterized by the purple colouration of the leaves.

Recommendations

The package of recommendation mentioned for jasmine also holds good in this case as well except for the fact that it is enough that half of the recommended chemicals is given.

DIAGNOSIS OF NUTRITIONAL AND PHYSIOLOGICAL DISORDERS IN COLE CROP VEGETABLES AND THEIR REMEDIAL MEASURES

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

The nutritional or physiological disorders which would occur at different phonological stages of crop growth are caused either due to deficiency or toxicity and unfavourable environmental conditions in which the crops are cultivated. In order to maintain or increase the yield of crops, these deficiencies and disorders need corrective measures and following are the common methods to correct these symptoms in vegetable crops. Apart from there normal deficiency symptoms, sometimes complex symptoms caused due to more than one or some time the deficiency of some nutrients may lead to pathogens attack, and in such cases, the exact disorders should be diagnosed and necessary correction measures are followed. In addition to these above mentioned nutritional deficiencies and disorders, some specific nutrient disorders would occur only in specific crops. In other words, when a particular element is not available to the specific crops for their metabolic functions, due to disruption of metabolic activities, the crops would manifest certain deficiency symptoms and these crops are called as 'indicator plants'. The common and very specific nutritional disorders and their symptoms occur in some of the temperature (cole) vegetables are listed below:

Deficiency	**Soil application (kg ha^{-1})**	**Foliar spray (per cent)**
Nitrogen	i.Application of 50 kg urea (or)	Spraying of 1% urea
	ii.Application of 50 kg DAP	
Phosphorus	Application of 25-50 kg DAP (or) 50 µg SP	Spraying of 2% DAP or SP
Potassium	Application of Calcium chloride at 60 to 80 kg ha-1 or CAN at 50 kg	Spraying of Kcl 1%
Calcium	Application of Calcium chloride at 60 to 80 kg ha-1 or CAN at 50 kg	Spraying of 0.5% CaNO3 (or) CaCl2 (or) Calcium ammonium nitrate
Sulphur	Application of Gypsum at 50 kg ha-1	Spraying of 0.5% gypsum

Magnesium	Application of magnesium sulphate 25-50 kg ha-1	Spraying of 0.5% $MgSO_4$ + 1% urea
Iron	Application of ferrous sulphate 50-100 kg	Spraying of $FeSO_4$ 1 to 2%
Zinc	Application of zinc sulphate 25 kg	Spraying of 0.5% $ZnSO_4$
Manganese	Application of 25 kg manganese sulphate	Spraying of 0.5% $MnSO_4$
Copper	Application of 5 to 10 kg copper sulphate	Spraying of 0.1% $CuSO_4$
Boron	Application of sodium tetraborate or borax at 10 kg	Spraying of borax at 0.05%
Molybdenum	Application of sodium molybdate or ammonium molybdate at 0.5% to 1.0 µg	Spraying of 0.01% sodium or ammonium molybdate

1. Cabbage

The savoy cabbage is highly sensitive to molybdenum deficiency. 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

Cauliflower is one of the most important cole crops of India. It requires high nitrogen, boron and molybdenum.

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 Misra, 1974).

Calcium: Physiological disorder 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 cause 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 (Millikand nd Hanger, 1966). Several calcium deficiency in this also occur 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 disorder (a) Forking: Damage to the tap root of carrot seedlings cause forking (*i.e*. splitting).

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 abscissisc 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: Leaves 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 / pot 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

Blachheart 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).

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.

Boron: Brown cracking caused due to boron deficiency.

Ex. No.14. Identification of physiological and nutritional and correction measures in vegetable crops

Vegetables are very often subjected to nutrient stresses because of their rapid growth, high nutrient requirement and intensity of production. The requirement of nutrients for vegetable crops is considerably higher than that of field crops. It has been observed that nitrogen should be high in fertilizers for leafy foliage crops, that phosphorus should be high for fruit crops and potash high for root crops.

Symptoms of nutrients deficiencies that develop in vegetable crops grown in the field often vary from those produced in plants grown under controlled greenhouse conditions. Disease and insects often produce plant symptoms closely resembling those resulting from nutrient deficiencies.

Cauliflower

"Brown heart disorder"

Boron deficiency in cauliflower, turnip, radish, cauliflower and other root crops, commonly cause what is known as brown heart. It is manifested first by dark spots on the roots, usually on the thickest parts. The plant gradually becomes stunted or dwarfened. The leaves are smaller than normal and fewer.

In the case of cauliflower, the flower head will show hollow stem and bronzing due to the decay of the core tissues of the stalk.

Recommendations

To ameliorate this disorder, foliar spray of 500 g of borax along with 100 g of urea and 100 ml of soap solution dissolved in 100 lit of water has to be taken up in an acre of land. Spray is to be repeated two to three times at an interval of 20 days.

Chillies

"Little leaf with intervenial chlorosis"

Symptoms

The chillies crop has high affinity for zinc nutrition and hence large amount of zinc is taken up by the plant. If zinc is not replenished periodically, the crop is prone to suffer from zinc deficiency severely.

The entire foliage of the crop will be reduced in size with interveinal chlorosis, when zinc is in short supply and the plant will present a stunted growth.

Recommendations

Foliar application of zinc sulphate @ 500 g and urea @ 100 g dissolved in 100 lit of water along with 100 ml soap solution is to be given two to three times at an interval of 20 days depending upon the extent of severity.

Foliar application has to be supplemented with soil application of zinc sulphate @ 8 kg/acre is given basally during last ploughing of the field.

Brinjal

"Iron Chlorosis"

Symptoms

Young as well as older leaves will exhibit chlorotic symptoms in the form of yellwing. So there will be a decline in photosynthesis due to which fruit yield will be greatly reduced.

Recommendations

Foliar spray of solution containing 500g of ferrous sulphate and 100 g of urea dissolved in 100 lit of water added with 100 ml of soap solution for an acre of land is taken up two or three times at an interval of 20 days depending upon the degree of severity.

Coconut

"Pencil point disease"

This is a physiological disorder affecting coconut palms to a great extent in areas such as Tanjore, Thiruvarur, Pattukottai, etc. The onset of this disorder is brought about

by blockage of vascular bundles which may lead to disruption in the transpiration process.

The typical symptom is characterized by the tapering of truck towards the crown, the fronds will become smaller, rigid and erect. The outer fronds will first show yellowing and drop off. As a result, the number of fronds will be reduced. The nuts will be fewer in number and smaller in size; most of the nuts will be hollow. Thus the yield of nuts is severally affected once this disorder sets in the coconut palm.

Recommendations

Along with the recommended fertilizer dose, 225 g each borax, zinc sulphate, manganese sulphate, ferrous sulphate, copper sulphate and 10 g of ammonium molybdate may be dissolved in 10 litres of water and poured in the basin of 1.5 m radius from the trunk of the palm.

FLOWER CROPS

Jasmine

"Nematode induced chlorosis"

Symptoms

The foliage will exhibit interveinal chlorosis as a result of iron deficiency in the soil, when the plant is infested with nematode the iron and other nutrient availability by translocation to the plant tissues is hampered due to blockage of vascular bundles as a result it is made to suffer from shortage of iron and other nutrients as also water. The leaves will have fade yellow colouration; the growth of the plant is stunted which may eventually lead to the wilting of the entire plant. The flower yield will be substantially reduced.

Amelioration

The iron chlorosis is a common occurrence in jasmine as many of the areas where it is widely grown has been infested with nematode. So, control of nematode infestation has to be taken up on priority basis.

After digging the soil around the tree, the following are applied to the soil : 10 g of Temic or 20 g of Furadon, 250 g of Neem cake and 50 g of murate of potash. It is followed by irrigation and application of 0.1% emison solution. After one week, foliar application of 500 g of ferrous sulphate and 100g of urea along with 100 ml of soap solution mixed in 100 lit of water is done two to three times at 20 days interval depending upon the extent of severity.

Rose

Failure of bud to blossom is a serious physiological disorder in rose which affects the marketability of the rose flower.

Recommendations

The fungicide Bevistin is taken @ 100 g and dissolved in water and the clear solution is further used for dissolving 1 kg super phosphate along with 100 g urea and 100 ml of soap solution. The entire mixture is added to water the total volume of which is made up to 100 litres. This volume of water is sufficient for spray in an area of one acre of land.

Crossandra

"Nematode Induced Chlorosis"

Symptoms

Crossandra is also affected by nematode induced chlorosis just like jasmine. In this crop, the typical symptom is characterized by the purple colouration of the leaves.

Recommendations

The package of recommendation mentioned for jasmine also holds good in this case as well except for the fact that it is enough that half of the recommended chemicals is given.

Diagnosis of nutritional and physiological disorders in cole crop vegetables and their remedial measures

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

The nutritional or physiological disorders which would occur at different phonological stages of crop growth are caused either due to deficiency or toxicity and unfavourable environmental conditions in which the crops are cultivated. In order to

maintain or increase the yield of crops, these deficiencies and disorders need corrective measures and following are the common methods to correct these symptoms in vegetable crops. Apart from there normal deficiency symptoms, sometimes complex symptoms caused due to more than one or some time the deficiency of some nutrients may lead to pathogens attack, and in such cases, the exact disorders should be diagnosed and necessary correction measures are followed. In addition to these above mentioned nutritional deficiencies and disorders, some specific nutrient disorders would occur only in specific crops. In other words, when a particular element is not available to the specific crops for their metabolic functions, due to disruption of metabolic activities, the crops would manifest certain deficiency symptoms and these crops are called as 'indicator plants'. The common and very specific nutritional disorders and their symptoms occur in some of the temperature (cole) vegetables are listed below:

Deficiency	**Soil application (kg ha^{-1})**	**Foliar spray (per cent)**
Nitrogen	i.Application of 50 kg urea (or)	Spraying of 1% urea
	ii.Application of 50 kg DAP	
Phosphorus	Application of 25-50 kg DAP (or) 50 µg SP	Spraying of 2% DAP or SP
Potassium	Application of Calcium chloride at 60 to 80 kg ha-1 or CAN at 50 kg	Spraying of Kcl 1%
Calcium	Application of Calcium chloride at 60 to 80 kg ha-1 or CAN at 50 kg	Spraying of 0.5% CaNO3 (or) CaCl2 (or) Calcium ammonium nitrate
Sulphur	Application of Gypsum at 50 kg ha-1	Spraying of 0.5% gypsum
Magnesium	Application of magnesium sulphate 25-50 kg ha-1	Spraying of 0.5% MgSO4 + 1% urea
Iron	Application of ferrous sulphate 50-100 kg	Spraying of FeSO4 1 to 2%

Zinc	Application of zinc sulphate 25 kg	Spraying of 0.5% ZnSO4
Manganese	Application of 25 kg manganese sulphate	Spraying of 0.5% MnSO4
Copper	Application of 5 to 10 kg copper sulphate	Spraying of 0.1% CuSO4
Boron	Application of sodium tetraborate or borax at 10 kg	Spraying of borax at 0.05%
Molybdenum	Application of sodium molybdate or ammonium molybdate at 0.5% to 1.0 µg	Spraying of 0.01% sodium or ammonium molybdate

1. Cabbage

The savoy cabbage is highly sensitive to molybdenum deficiency. 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

Cauliflower is one of the most important cole crops of India. It requires high nitrogen, boron and molybdenum.

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 Misra, 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.

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

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

8. 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

Blachheart 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).

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.

Boron: Brown cracking caused due to boron deficiency.

Ex.No.15. Diagnosis of deficiencies through rapid tissue testing

The nutrient requirement for growth of the plants at different stages is an important factor for crop productivity. The nutrient status of the plant at a particular stage should be analysed so as to supply the deficient nutrient in proper quantity for good crop yield. When nutrient deficiency problem arises, the plants will manifest symptoms of deficiency for example**, whiptail** of cauliflower (Mo deficiency), **little leaf disease** (Zn deficiency) in rice.

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
Maize	Stem, midribs	Leaf blade	Leaf blade
Soybean	Petiole	Leaf blade	Petiole
Black gram	Petiole and lamina	Leaf blade	Leaf blade
Cotton	Petiole	Petiole	Petiole
Papaya	Petiole	Petiole	Petiole
Tomato	Petiole	Petiole	Petiole
Banana	Lamina	Lamina	Lamina

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.

Dark blue - Sufficient

Light blue - Slightly deficient

No color - Highly deficient

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.

Dark blue	-	Rich in phosphorus
Light blue	-	Moderately sufficient
Green or bluish green	-	Deficient
No color or yellow	-	Highly deficient

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.

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.

Clear reddish brown	-	Insufficient
Deep canary yellow turbidity	-	Sufficient

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

Colorless	-	Insufficient
Greenish white turbidity	-	Sufficient

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.

Straw yellow - Insufficient

Salmon pink color - Sufficient

Chloride

Reagents

N/50 silver nitrate

Concentrated Nitric acid

Procedure

To 2ml of Morgan's reagent extract, 2ml of N/50 $AgNO_3$ and 3 drops of concentrated nitric acid are added and kept for color development.

Colorless - Insufficient

White turbidity - Sufficient

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 color development.

Brick red: Sufficient

Faint color: Deficient

Manganese

Sensitive test for deficient leaves

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 shaken vigorously and observed for color development.

Pale blue - Insufficient

Deep blue - Sufficient

Ex.No.16. Seed viability by tetrazolium test

Seed physiology

Structure of seed

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.

Examples of Albuminous Seeds

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

Examples of Exalbuminous Seeds

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

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.

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.

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

S.No	Crop	% of viable seeds (Stained)	% of non-viable seeds (unstained)

Ex.No.17. Practical Examination

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