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ACHARYA N.G. RANGA AGRICULTURAL UNIVERSITY

Department of Plant Physiology

Detailed Lecture Outlines

1. Course No : PPHY 161

2. Course title : **CROP PHYSIOLOGY**

3. Credit hours : 3 (2+1)

4. General objective : To impart knowledge to the students on different plant metabolic processes and their functions in plants.

5. Specific objectives

a) Theory

By the end of the course, the student will be able to

i) Study the growth and development of plants

ii) Study the effect of nutrients and Plant growth regulators, and their applications in agriculture.

iii) Understand the physiology of seeds and fruit ripening

b) Practical

By the end of the practical exercises, the students will be able to

i) Understand various plant metabolic processes, at different stages of plant growth, which lead to development.

A) Theory Lecture Outlines

1. Introduction – definition of crop physiology – importance in agriculture and horticulture.
2. Seed physiology – seed structures – development of embryo, endosperm, perisperm and seed coat – morphological, physiological and biochemical changes during seed development.
3. Seed physiology – physiological maturity – morphological and physiological changes associated with physiological maturity in crops with examples – harvestable maturity – seed viability and vigor – factors affecting seed viability and vigor.
4. Seed physiology – methods of testing seed viability and vigor – germination – utilization of seed reserves (carbohydrates, fats and proteins) during seed

germination – morphological, physiological and biochemical changes during seed germination – factors affecting seed germination.

5. Growth and development – definition – types of growth – determinate and indeterminate growth – monocarpic and polycarpic species with examples- initiation and development of vegetative and reproductive structures.
6. Growth and development – measurement of growth – growth analysis – growth characteristics – definitions and mathematical formulae.
7. Crop water relations – physiological importance of water to plants – water potential and its components-Importance of water potential-Active and passive uptake of water-measurement of water status in plants.
8. Crop water relations – transpiration – definition – significance – structure of stomatal complex in monocots and dicots – role of stomata in transpiration – transpiration in relation to crop productivity – Water Use Efficiency (WUE) – WUE in C_3, C_4 and Crussulacean Acid Metabolism (CAM) plants – WUE of major field crops – factors affecting WUE.
9. Photosynthesis – energy synthesis – Cyclic and Non cyclic photo phosphorylation- carbon dioxide fixation – C_3 pathway.
10. Photosynthesis – carbon dioxide fixation – C_4 and CAM pathways – methods of measuring photosynthesis.
11. Photosynthesis – photorespiration – factors affecting photosynthesis (light, carbon dioxide, temperature, water stress, water logging, salinity, weeds / weedicides etc.)
12. Photosynthesis – photosynthetic efficiency – significance of C_3, C_4 and CAM pathways – relationship of photosynthesis and crop productivity.
13. Translocation of assimilates – phloem loading – apoplastic and symplastic transport of assimilates – mechanism of phloem transport – phloem unloading
14. Source and sink concept – dry matter partitioning – harvest index of crops.
15. Respiration and its significance – importance of glycolysis, Tricarboxylic Acid (TCA) cycle and Pentose Phosphate Pathway.
16. Respiration – interrelationship of respiration and photosynthesis – growth respiration and maintenance respiration – alternate respiration – salt respiration – wound respiration – measurement of respiration.
17. Nutriophysiology – definition – essential elements – criteria of essentiality of elements – classification of plant nutrients based on their Biochemical role and physiological function.
18. Nutriophysiology – physiology of nutrient uptake – active and passive uptake of nutrients – functions of N, P, K, Ca and Mg.
19. Nutiophysiology – functions of Fe, Zn, Mn, Cu, B, Mo, Cl, Na and Si - their mobility in phloem
20. Nutriophysiology – Deficiency and toxicity symptoms of plant nutrients
21. Nutiophysiology – foliar nutrition – hydroponics – solution and sand culture

22. Photoperiodism and flowering – importance of photoperiodism – classification of plants based on photoperiodic responses – perception of photoperiodic stimulus – Biological clock.
23. Photoperiodism – phytochrome – flowering hormones – vernalization and flowering – importance of vernalization in relation to crop productivity.
24. Plant growth regulators – occurrence, biosynthesis, mode of action and physiological role of auxins
25. Plant growth regulators – occurrence, biosynthesis, mode of action and physiological role of gibberellins
26. Plant growth regulators – occurrence, biosynthesis, mode of action and physiological role of cytokinins
27. Plant growth regulators – occurrence, biosynthesis, mode of action and physiological role of Abscisic Acid (ABA)
28. Plant growth regulators – occurrence, biosynthesis, mode of action and physiological role and ill effects of ethylene
29. Plant growth regulators – novel plant growth regulators – commercial application of plant growth regulators in agriculture and horticulture
30. Senescence and abscission – definition – classification – theories of mechanism and control of senescence – physiological and biochemical changes and its significance – abscission and its relationship with senescence.
31. Post harvest physiology – seed dormancy – definition – types of seed dormancy – advantages and disadvantages of seed dormancy – causes and remedial measures for breaking seed dormancy with examples – optimum conditions of seed storage – factors influencing seed storage- International Seed Testing Association (ISTA) standards
32. Post harvest physiology – fruit ripening – metabolic changes during fruit ripening – climacteric and non-climacteric fruits – hormonal regulation of fruit ripening (with etherel, Chloro Choline Chloride (CCC), polaris and paclobutrazol) – use of hormones in increasing vase life of flowers

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1) Bidwell, R.G.S 1995, *Plant Physiology,* Macmillan Publishers Co., New York .

2) Delvin, R.M. and Witham, F.H. 1986, *Plant Physiology* CBS Publishers & Distributors, Delhi.

3) Frank, B. Salisbury and Cleon, W. Ross, 2005. *Plant Physiology*, CBS Publishers & distributors, Delhi.

4) Gardener, P., Brent Pearce, R. and Roger, L. Mitchell, 1985. Physiology of Crop Plants. Jodhpur Scientific Publications, Jodhpur.

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6) Lincoln Taiz and Eduardo Zeiger 2006, Plant Physiology, Sinauer Associates, Inc. Publishers, Sunderland, Massachusetts.

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Lecture-1

IMPORTANCE OF CROP PHYSIOLOGY IN AGRICULTURE AND HORTICULTURE

Genetic potential of a plant and its interaction with environmental factors decides its growth and development by influencing or modifying certain internal processes. Plant physiology studies about these internal processes and their functional aspects. It helps to understand various biological processes of the plants like Photosynthesis, respiration, transpiration, translocation, nutrient uptake, plant growth regulation through hormones and such other processes which have profound impact on crop yield.

Crop: is a group of plants grown as a community in a specific locality and, for a specific purpose.

1.1 Crop Physiology- Definition :

Crop physiology is the study of the ways in which plant physiological processes are integrated to cause whole plant responses in communities.

The subject matter of crop physiology includes the ways in which the knowledge of plant physiology is applied for better management of crops.

1.2 A BRIEF HISTORY OF CROP PHYSIOLOGY:

1771- Joseph priestly : Plants could regenerate oxygen in the atmosphere

1779- Ingenhouz : Studied the role of light in Photosyntheis

1804- De Saussure: Plant uptake of mineral nutrients and nitrates from soil**.**

1837- Boussingault: Nitrogen uptake by plants both from soil and atmosphere

1865- Julius Sachs :published '*Experimentelle Pflanzenphysiologi*' . By this time all branches of plant physiology were completely established, but their development in the next fifty years showed little concern with crop productivity and yield, except in the area of mineral nutrition. In this period essentiality and role of many mineral nutrients was established. Towards the end of that period herbicide physiology also became prominent.

W.LBalls (1915): Crop physiology, with the aim of understanding the dynamics of yield development in crops, really began with the work of W.L.Balls. Along with

Holton he analysed the effects of plant spacing and sowing date on the development and yield of Egyptian Cotton plants with in crop stands, **not in isolated plants**. It was from his work the term 'crop physiology' came in to existence. From then onwards, various scientists have started applying the advances in physiological knowledge for better crop management.

1924- In England- a rapid development of the methods of growth and yield analysis by different investigators (V.H.Blackman, F.G.Gregory, G.E.Briggs etc.) was started. With the development of various methods of growth analysis, they started explaining 'the physiology of crop yield'

1947: The concept of LAI (Leaf area index) was developed by **D.J.Watson**. This index has provided a more meaningful way of analyzing growth in crops, and stimulated renewed interest in crop physiology.

1950's: Studies on photosynthetic rate of the leaf and the loss of photosynthates by respiration was studied by the development of 'Infra Red Gas Analysis (**IRGA**)'method. This method has facilitated the estimation of short term rates of Photosynthesis and respiration by crops in the field.

1953: Monsi and Saeki explained about the manner of light interception by the crop canopy with their concept of light interception coefficient.

1963: Hesketh and Moss showed that photosynthesis by leaves of Maize, Sugarcane and related tropical grasses could reach much higher rates, with less marked light saturation, than leaves of other plants(This was the starting point for research to find other photosynthetic CO_2 fixation path ways like C_4, and CAM Mechanisms). The differences in pathway are associated with differences in photosynthetic rate, in response to light intensity, temperature and oxygen level, in photorespiration, in leaf anatomy and chloroplast morphology, in rate of translocation, and in the efficiency of water use, which can have profound effects on the physiology of yield determination.

Later on several research works were carried out to understand the processes like translocation of food materials, their partitioning towards economic yield, storage mechanisms, physiology of flowering, effect of stressful environmental factors on crop growth and development, role of plant growth regulators in increasing the crop productivity etc. All these areas have enriched the knowledge of physiological processes and their role in deciding the crop yield.

1.3 IMPORTANCE OF CROP PHYSIOLOGY IN AGRICULTURE AND HORTICULTURE:

Many aspects of Agriculture and Horticulture can benefit from more intensive research in plant physiology to provide practical solutions in agriculture and horticulture. Understanding the physiological aspects of seed germination,

seedling growth, crop establishment, vegetative development, flowering, fruit and seed setting and crop maturity provides a reasonable scientific base for effective monitoring and beneficial manipulation of these phenomenon's. Studying this phenomenon with a view to develop better crop management practices forms the subject matter of crop physiology. The importance of physiology in agriculture and horticulture can be seen from the following examples;

- Seed is the most important input in agriculture. Germination of seed and proper establishment of seedling depends upon various internal and external factors. Knowledge in **Seed physiology** helps in understanding of different physiological and morphological changes that occur during germination. Any deviation in these processes causes Seed dormancy. The dormant condition of the seed bars immediate use of harvested seed for next crop which is important in intensive agriculture. By understanding the causes and effects of this problem, Crop physiologists have come up with different methods of breaking the seed dormancy.

  Example: When ever Paddy is used as a seed material in the very next season it is recommended to treat the seed either with HNO_3 or with GA.

- The first prerequisite for higher yields in crops is high **total dry matter production** per unit area. High dry matter production is a function of optimum leaf area (Optimum leaf area Index) and Net Assimilation rate.

  (**CGR = LAI X NAR**).

  Example: Pruning operation in horticultural crops like Mango is done based on this principle of proper canopy management for better photosynthesis.

- Total amount of dry matter produced less the photosynthates used in respiration is the net product of photosynthesis. Economic yield depends on how the dry matter is distributed among different organs of the plant. Partition of total dry matter amongst the major plant organs is of interest to the farmers as they are more interested in its partition towards economic yield.

  Example: excessive vegetative growth period in Ground nut produces less number of Pods as the reproductive period gets constricted. Thus, groundnut varieties with relatively extended period of reproductive growth are desirable.

- The use of **herbicides** to kill unwanted plants is widespread in modern agriculture. Majority of Herbicides -about half of the commercially important compounds—act by interrupting photosynthetic electron flow.(Ex. Paraquat, diuron). When the electron transport is blocked it virtually stops light reaction of photosynthesis. When light reaction is stopped the dark reaction does not happen and thus CO_2 is not fixed as carbohydrate. Therefore the weed is killed by starvation.

- Nutriophysiology is yet another important area to under stand crop physiology. For the healthy growth of a crop around 16 essential elements are required. Knowledge of **nutriophysiology** has helped in identification of essential nutrients, ion uptake mechanisms, their deficiency symptoms and corrective measures. It also helps to check the toxicity symptoms of various nutrients.

  Examples: Zinc deficiency leads to Khaira disease in Rice. This can be controlled either by soil or by foliar application of Zinc sulphate.

- Response of plant to the relative length of day and night is called as **photoperiodism**. This concept was used to choose photo insensitive varieties. The semi dwarf Rice varieties that have revolutionized Indian agriculture, are lodging resistant, fertilizer responsive, high yielding and **photo insensitive**. Photo insensitivity has allowed rice cultivation in non-traditional areas like Punjab. Even in traditional areas rice-wheat rotation has become possible only due to these varieties.

- Plants can regulate their growth through internal growth mechanisms involving the action of extremely low concentrations of chemical substances called Plant growth substances, phytohormones or **Plant growth regulators**. The regulation of flowering, seed formation and fruit setting has been controlled through the application of different hormones at the appropriate time of plant height and age.

  Example:Commercial preparations of rooting compounds are available (Indole Buteric Acid @250 ppm) that promote callus and root formation which can improve establishment from stem cuttings

- Indian agriculture being predominantly rain fed in nature, development of drought resistant varieties is very important. Root zone depth, density of roots, plant water potential, relative water content, water use efficiency, xerophytic characters of leaves etc. are some of the characters helped to bred drought tolerant varieties and to develop efficient irrigation management practices (sprinkler and drip irrigation).

  Among Several physiological approaches, transpiration efficiency or **water use efficiency** is the most dependable trait, which is “the amount of dry matter produced per unit amount of water transpired”. The importance of water use efficiency (WUE) in influencing grain yield under water limited conditions can be explained by the following model give by passioura.

  Grain Yield = T x TE x HI

  Where T = Total transpiration by the crop canopy

  TE = Transpiration Efficiency or WUE

  HI = Harvest Index (Economic Fraction of Dry matter)

This relationship provides an analytical tool to select the genotype with high levels of T and TE.

- **Post harvest losses** of agriculture and horticulture are causing a great distress to farming community. Moisture and temperature are the two important factors causing physiological changes that reduce the post harvest quality of grains. Control of moisture content and maintenance of low temperatures have proved effective in storage of grains. Being perishable in nature the magnitude of post harvest loss is comparatively higher in horticultural crops.

  Example: In recent years a method called '**modified atmospheric storage**' was developed for prolonged post harvest life of fruits and vegetables.

Shelf life of cut flowers can be increased by application of kinetin (cytokinin). This will reduce the burst of ethylene and thus reduces the rate of senescence.

Thus, physiological understanding of crop plants provides the fundamental scientific base about various aspects of metabolism, growth and development. This is immensely important for crop improvement or technology improvement in agriculture or horticulture.

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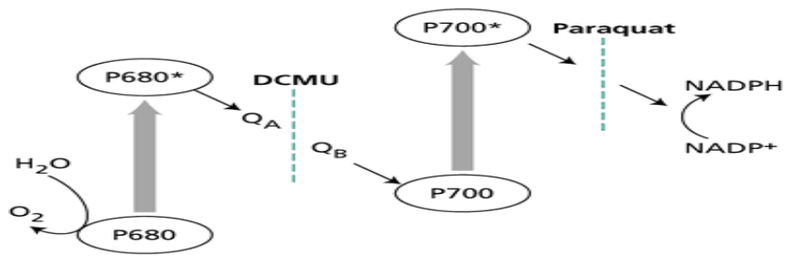
ANNEXURE



DCMU: Di chloro phenyl di -methyl urea (chemical name of Diuron)

Lecture-2

SEED PHYSIOLOGY

Much of the success on modern agriculture depends on the availability of high quality seeds with good genetic potential and proven performance in germination, emergence, and vigorous Vegetative growth.

Seed: Seed is a fertilized mature ovule containing an embryonic axis (embryo), stored food material(endosperm) and a protective covering (seed coat or testa).

2.1 Seed structures:

Living embryo, the most important part of seed, consists of two structures (i) embryonic axis and (ii) cotyledon(s). The embryonic axis is composed of three parts namely (i) radicle (embryonic root), the hypocotyls (point of attachment of cotyledons) and (iii) plumule (the shoot apex with the first true leaves). The three parts of embryonic axis are easy to identify in dicots, but in monocots (especially in the family Gramineae) their identification is difficult. In monocots, there is only one cotyledon, which is reduced and modified to form the **scutellum**. The basal sheath of cotyledon is elongated to take the shape of **coleoptile**, while in some cases (e.g., maize), the hypocotyls is modified to form mesocotyl. The base of hypocotyls sheathing the radicle is termed as **coleorhiza**.(Figure.1)

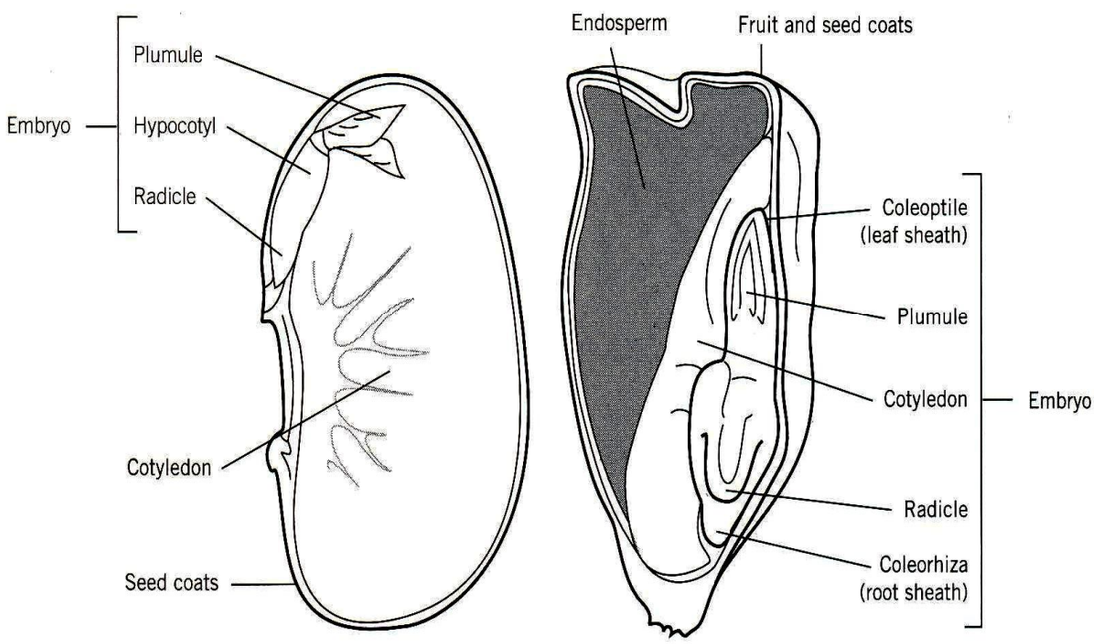


Figure.1 Seeds of common bean (*Phaseolus vulgaris*) (left), and Maize (*Zea mays)* (Right), a monocot, a dicot, showing the embryo.

**Figure*.1 is from reference .5*

2.2 Seed development: There are many variations in the pattern of seed development when the entire plant kingdom is considered. However, the general process of seed development is almost similar. This includes

- EMBRYO DEVELOPMENT
- ENDOSPERM DEVELOPMENT
- SEED COAT DEVELOPMENT

2.2.1 EMBRYO DEVELOPMENT:

Embryo is a connecting link between two generations of a plant and provides a continuity of genetic material. In most of the Angiosperms, the embryo and endosperm start their development as soon as double fertilization is over. Embryo is diploid in its genetic constitution and is developed from the zygote formed by fertilization between egg cell & one of the sperm nuclei. The embryo or embryonic axis represents the life of a plant in miniature.

The size and shape of embryo varies among the plant species. In Monocots (Ex.wheat) where the endosperm is well developed, the embryo occupies less space of the seed than in dicot species. Exceptionally, the cotyledons of Castor are thin and flattened (leaf like structure) since endosperm acts as a storage structure.

2.2.2 ENDOSPERM DEVELOPMENT:

Endosperm serves as the principal nutritive source (food material) for the embryo during seed development and germination. Endosperm development precedes embryo development. Endosperm is developed from primary endospermic nucleus which is a resultant of fusion between two polar nuclei and one of the sperm nuclei (which is formed from the division of generative nucleus).Thus, the endosperm of Angiosperms is Triploid (3n) and its development follows any one of the following three types.

A) Nuclear endosperm: In the nuclear type of endosperm development, the primary endosperm nucleus divides by repeated mitotic free nuclear divisions without the formation of walls. Ex. Wheat.

B) Cellular endosperm: In the cellular type of endosperm development, the first nuclear division of the primary endosperm nucleus is followed by the formation of either a longitudinal or transverse cell wall in the central cell. Subsequent nuclear divisions and wall formations result in the formation of a cellular type of endosperm, *e.g., Petunia, Datura, Balsam,* etc.

C) Helobial endosperm: It is an intermediate form of nuclear & cellular endosperm development. Helobial type of endosperm development is prevalent in monocotyledons.

In monocots development of endosperm reaches to its maximum by the time of maturity. However, in dicots endosperm development is not so conspicuous because of its continuous utilization by the embryo or it may remain as a small part of the seed (e.g. Groundnut and Soyabean).

Seeds with well developed endosperm are called endospermic or **albuminous** while those with small amounts of endosperm are non- endospermic or **exalbuminous**.

2.2.2.1 EXAMPLES OF ENDOSPERMIC SEEDS:

Monocots: Rice, Wheat

Dicots: Castor, opium

Legumes: Fenugreek

NON ENDOSPERMIC SEEDS

Monocots: Orchids, Water plantain

Dicots : Grams, Peas, Beans

2.2.2.2 ALEURONE LAYER:

In cereals and endospermic legumes the majority of cells in endosperm are non-living. However, the living cells are present in the outermost layer of endosperm and this layer is known as aleurone layer. During the development of endosperm, cells in aleurone layer are filled with protein granules and most of them are enzymatic in nature. Consequently this layer functions both as a storage tissue and also secretes enzymes of hydrolytic nature (like α-amylase) necessary for germination.

2.2.2.3 PERISPERM : In some of the plant species like coffee and Pig weed, endosperm is absent and the perisperm acts as a storage tissue. Perisperm (2n) is developed from maternal nucellus.

2.3 SEED COAT DEVELOPMENT:

During seed maturation the outer structure of ovules namely integuments undergo marked recognition to form the seed coat (testa). The seed coat acts as a protective barrier between the embryo and the external environment. The color and the texture of the seed coats vary from species to species and with in the species.

Almost all seeds bear a scar like point called **hilum**. This is the point at which a seed remains joined with the funicles. The **micropyle,** which is a small hole at one end of hilum, is present in seed coats of many species. Sometimes hairs or wings are also present in testa; these structures help in seed dispersal, e.g., in willow, lily etc. Seed coat may also bear outgrowths of the hilum region, viz, **strophiole** which

controls movement of water into and out of seed, and the **aril**, which contain chemicals. For example, the aril of nutmeg is used as a source of spice mace. In castor bean, the aril, which is associated with micropyle, is called **caruncle.**

2.3 MORPHOLOGICAL AND PHYSIOLOGICAL CHANGES DURING SEED DEVELOPMENT:

1. Soon after fertilization the embryo starts developing as soon as endosperm completes its development. This phase of development is characterized by **cell division** and **differentiation**.
2. Several **sub-cellular organelles** *viz.,*Plastids, Mitochondria, Ribosomes, Golgi complex and Endoplasmic reticulum become recognizable immediately after cell formation.
3. The sub-cellular organelles undergo changes in their arrangement and activities. They also participate in the processes leading to the formation of embryo.
4. The embryo shows variation in shape depending upon its location (Folded cotyledons in Cotton, Bent in Fabaceae, Spatulate in Lamiaceae and peripheral in Caryophyllaceae)
5. Variation in the ratio of embryo and endosperm
   - In dicots the developing embryo utilizes endosperm completely. Therefore the ratio of embryo to endosperm is more.
   - In monocots the ratio of embryo to endosperm is less.

2.4 BIOCHEMICAL CHANGES DURING SEED DEVELOPMENT:

1. The seed increases in its weight due to synthesis and deposition of seed reserves like starch, fat, protein, nucleic acid and phytine.
2. Starch is the major **carbohydrate** that increases rapidly in the endosperm.
3. As development proceeds, the proteins (nitrogen) accumulate in the cotyledons of the embryo.
4. The synthesis of proteins in the embryonic axis will be accompanied by increase in RNA & DNA (nucleic acids) due to formation of new cells.
5. There will be simultaneous accumulation of storage **lipids** (lipid bodies) in the cell membranes between phospholipid layers.
6. **PHYTIN**- a rich source of Phosphorus is deposited as **myo-inositol hexa phosphoric acid**. This is found in the endosperm and aleurone cells of many seeds. (During germination phytin is hydrolyzed by the enzyme phytase to give inositol, which participates in numerous biosynthetic pathways essential to the developing embryonic axis)
7. Seeds also synthesize minor constituents like alkaloids (caffeine-in Coffee),Glycosides (Synigrin in Musturd), vitamins (Thiamin,Biotin and

ascorbic acid),inhibitors(Coumarin,ferulic acid and ABA)and hormones (Auxins,gibberellins etc.,)

8. There will be a change in the pigmentation of seed coat of peas which become colorless due to loss in the chlorophyll in chloroplasts.
9. Moisture content drops to 10-15% as a consequence of increase in depletion of seed reserves.
10. Thus, the above biochemical changes of seed indicate the complexity of development and maturity.

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Lecture-3

SEED PHYSIOLOGY

Crops are to be harvested at an optimum stage of maturity to get a qualitative and quantitative yield. Seed yield and its quality depends on number of factors .Time of sowing and harvesting stages are among the major considerations in deciding the seed quality and productivity.

3.1 PHYSIOLOGICAL MATURITY: Identification of harvesting stage in terms of easily measurable parameters is an important problem being faced by the farmers. **Physiological maturity or Biological maturity** in terms of average moisture content and maximum dry weight of seed is one such parameter on which farmers can depend to harvest maximum.

Physiological maturity can be defined as "**the stage at which the seed reaches to its maximum dry weight, viability and vigor capability**".

3.1.1 MORPHOLOGICAL AND PHYSIOLOGICAL CHANGES ASSOCIATED WITH PHYSIOLOGICAL MATURITY IN CROPS – SOME EXAMPLES:

Determination of physiological maturity in crops is very essential. Certain morphological and physiological changes in different parts of the plants can serve as a guideline to decide the physiological maturity. Effective and practicable guidelines in this regard help the farmers to reap a good harvest.

- Formation of black layer in placenta region near the point of kernel attachment in Maize and Sorghum.
- 35 to 40 days after anthesis in Sorghum.
- Change of internal pod walls in to brown color and attainment of pink colored seed coat in groundnut.
- Change of color of involucre bracts from green to yellow in Sunflower.
- Measuring BRIX reading (Total soluble solids)in sugar cane by 'Brix sugar hydro meter'. If the reading is 17 or more the crop is ready for harvest. Sucrose content of the cane and brix can be estimated with hand refracto meter.

3.2 HARVESTABLE MATURITY:

Generally, farmers decide the maturity period for harvesting in terms of crop duration from the time of sowing. However, this will change by the variety, soil fertility and climatic conditions. Such harvest, which coincides with the ripeness of seed beyond physiological maturity, is referred to as **harvestable maturity**. If the seeds are retained on the mother plant beyond physiological maturity, several morphological and physiological changes takes place in seeds and result in the decline of seed quality and yield. For example,

- Rice crop harvested at 15% seed moisture content instead of 21%, results in a yield reduction by 20 per cent.
- Harvesting the seed of Green gram crop after 4 weeks of pod initiation reduces the yield compared to a harvest of 2-3 weeks pod initiation.

3.3 SEED VIABILITY AND VIGOR

Generally the terms viability and vigor are misused at different levels. To most of the seed technologists viability means the capacity of a seed to germinate and produce a normal seed ling. In another sense viability denotes the degree to which a seed is active and possesses enzymes capable of catalyzing metabolic reactions needed for germination and seedling growth. In this context, a given seed may contain live and dead tissue and they may are may not be capable of germination i.e they may be viable or non viable.

Vigor is usually defined as that condition of active good health and robustness in seeds which upon planting, permits germination to proceed rapidly under a wide range of environmental or field conditions. A seed is said to be vigorous when it shows uniformity in germination and plant development under non-uniform conditions.

In most of the cases vigor and viability are probably the highest at the time of harvest and decrease gradually in storage. It is the vigor that declines first followed by viability (Fig.2). The potential with which a seed produces a healthy seedling depends on the vigor of seeds.

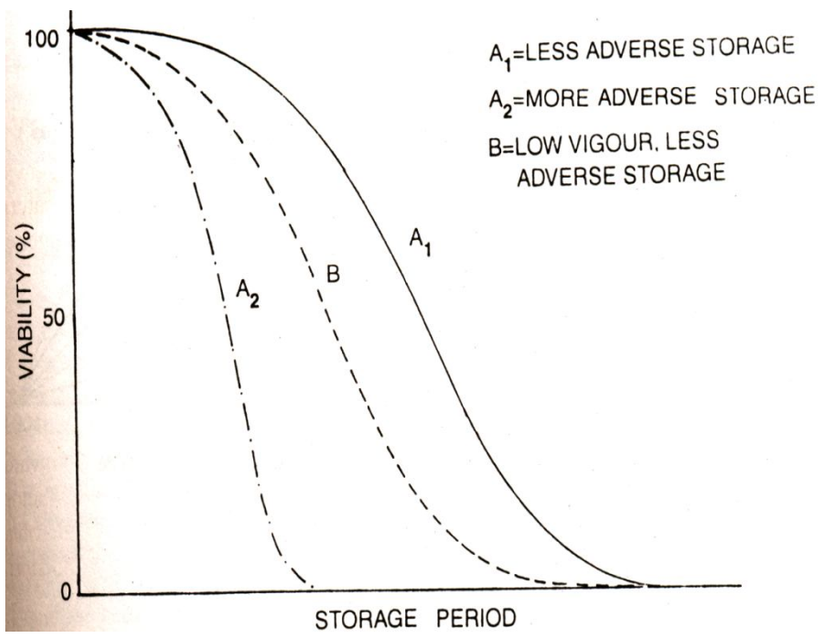

Figure.2 . Loss of viability and vigor during storage period

*Figure is from seed science and technology (by A.K.Joshi & B.D.Singh)

There are several factors affecting viability and vigor in seeds. Among them the most important are

3.4.1. Genetic make-up

Vigorous seeds produce seedlings of good health and natural robustness. The vigor of the seeds depends on the genome history of the individual seed and the environment in which it is sown.

Hybrid seeds normally possess greater vigor due to their inherent genetic make up. Genetic factors such as hard-seededness, resistance to diseases, and seed chemical composition influence the expression of seed vigor.

Viability is also influenced by the genetic make up of the seed. Some kinds of seeds are inherently short lived (e.g. onion, soybeans, ground nut etc.) and quickly loose their viability.

3.4.2 Time of harvest

Generally, seed viability and vigor are maximum at the time of physiological maturity. After physiological maturity seeds begin to deteriorate at varying rates depending on genetic factor and on the conditions of storage environment

3.4.3 Environmental factors during seed development

a) Temperature
b) Moisture availability
c) Soil fertility

Fluctuating temperature during seed formation and maturity , Pre-harvest rain and Pre harvest environment of high humidity and warm temperatures will affect seed viability and vigor.

The amount of moisture in the seeds is the most important factor influencing seed viability during storage. Generally if the seed moisture content increases storage life decreases. If seeds are kept at high moisture content the losses could be very rapid due to mould growth .very low moisture content below 4% may also damage seeds due to extreme desiccation in some crops.

Seeds developed under moisture stress, nutrient deficiency, extreme temperatures, etc. often result in light, shriveled seed or poor-vigor seeds.

3.4.4 Mechanical damage during threshing, Cleaning and transportation:

The rate of deterioration increases due to mechanical injury at the time of harvesting and processing.

3.4.5 Infestation by micro organisms of seed in storage:

Storage fungi adversely affect the seed by bringing down the seed viability, seedling vigor and also affect the chemical composition of seeds.

Aspergillus, Penicillium. Rhizopus were some most commonly occurring storage fungi which reduces seed germination and seedling vigor and causes a variety of symptoms on seedling.

The microflora activity is controlled by Relative Humidity temperature and Moisture content of seed.

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Lecture-4

SEED PHYSIOLOGY

4.1 METHODS OF TESTING SEED VIABILITY AND VIGOR

There are several tests to evaluate viability and vigor in seeds. The tests intended to determine viability and vigor must meet certain principles. The ideal test should be

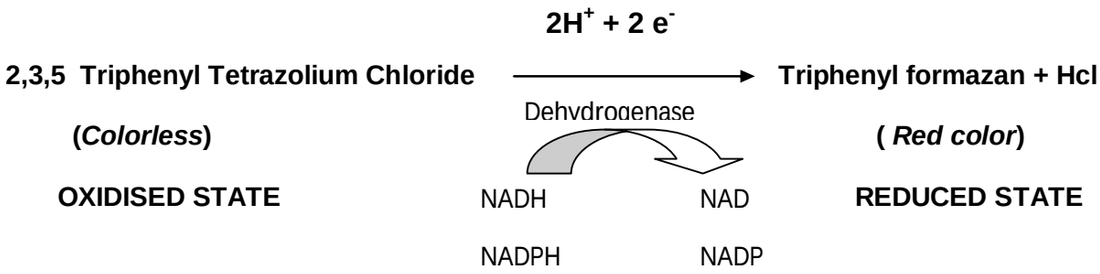
- ✓ Simple with out involving sophisticated equipment.
- ✓ Rapid- its procedure should take less time to give results.
- ✓ Effective in detecting minute as well as large differences.
- ✓ Equally useful for evaluating either individual seed or population of seeds.

4.1.1 VIABILITY TESTS:

A) Tetrazolium test: This test is often referred as a **quick test** since it can be completed with in hours. The test is usually based on measuring the activity of **dehydrogenase** enzyme in the tissues of embryo. It is conducted by using 2,3,5-Triphenyl Tetrazolium Chloride (**TTC**)solution.

Principle: Any living tissue must respire. In the process of respiration the enzyme dehydrogenase will be in a highly reduced state. When the seed is treated with colorless tetrazolium solution, the living tissue of the seed by virtue of respiration and having the dehydrogenase enzyme in a highly reduced state gives off hydrogen ions.

These hydrogen ions reduce the colorless tetrazolium solution in to red colored formazan. Thus, the tetrazolium test distinguishes between viable and dead tissues of the embryo on the basis of their relative rate of respiration in hydrated state.



B) Sulphuric acid test: This is usually a non- enzymatic test. The principle involved in this test is to distinguish the differential coloration of live versus dead tissue when exposed to sulphuric acid. The living portion of the cut surface of the seed develops **deep rose color** with in 5-10 minutes. Though this test takes less time, one must be careful in handling concentrated sulphuric acid.

A comparison between the above tests clearly indicates that tetrazolium test is more practicable, since it is quick and does not involve any risk.

4.1.2 VIGOR TESTS

a) Speed of Germination:

The speed of germination is an important aspect of vigor and provides a reasonably good index of vigor of any seed lot. Mathematically speed of germination is expressed as "**co-efficient of Germination**" (CG). Higher the value for speed of germination greater will be the vigor.

$$CG = \frac{100\ (A_1+A_2\ \text{----------}\ +A_x)}{A_1T_1+\ \text{----------}A_x\ T_x}$$

A: Number of seeds germinated

T: Time corresponding to A

X: No of days to final Count.

b) Exhaustion Test:

This test is more suitable for cereal seeds. The moist paper toweling or crape craft paper used in this test has to be rolled loosely along with the seeds placed on the printed line of the paper. Those seeds which exhaust their reserve food material and produce seedlings with roots and shoots extending more than two inches are said to be vigorous.

c) Respiration Test :

Respiration is one of the important processes common to all living organism. In seeds the rate of respiration and vigor are closely related. Hence, it is natural assumption that seeds with high vigor would have higher respiration rates . The differences in respiration rates have been used to distinguish between high, medium and low vigor seeds in maize, wheat and soybean. This test can be used to detect loss of vigor due to mechanical damage, chilling, desiccation and other causes.

Other tests like **accelerated aging test (AAT)**, **Brick gravel test**, **Electrical Conductivity test** are also used to estimate seed vigor.

4.2 Mobilization and Utilization of Seed Reserves

Once seeds have imbibed water and are hydrated, metabolic functions begin to accelerate if dormancy was previously broken. Enzymes to become active upon hydration and are involved in mobilization of seed storage reserves. Once storage reserves are breaking down, they are moved as small molecules from the source to those cells or organs that have a need for the product. In general meristematic and actively growing cells are energy and nutrient sinks. In germinating seeds, cotyledons and endosperm are sources.

Stored sources of energy and nutrients include:

Starch: Starch is found in amyloplasts. Starch serves as a source of reduced carbon for respiration and metabolism. Enzymes in involved in starch degradation are ***β*-amylase, α-amylase and Starch phosphorylase.**

Fats (oils): They are Stored in fat bodies. The high energy content of fats is broken down by β-oxidation in glyoxysomes and used for respiration and metabolism.

Storage proteins: Stored in protein bodies **.**Storage proteins are rich in amino acids with abundant nitrogen and sulfur. Amino acids rich in N are asparagine, glutamine and lysine. Sulfur-containing amino acids are methionine and cysteine. Storage proteins are broken down by aminopeptidases, carboxypeptidases, and endopeptidases.

4.3 MORPHOLOGICAL, PHYSIOLOGICAL AND BIOCHEMICAL CHANGES DURING SEED GERMINATION:

Seed germination is the resumption of active growth of the embryo that results in the rupture of the seed coat and emergence of the young plant. During the process of germination seeds undergo several metabolic changes. These changes can be summarized as follows.

- Initially the seeds imbibe water through the openings in the seed coat. Upon **imbibition** the cells become turgid, the seed increases in its size/volume and the seed coat becomes more permeable to diffusion of gases (O_2 and CO_2).
- There will be a change in the sub cellular organization of the embryo, endosperm and aleurone layer.
- Phytochrome becomes biologically active and triggers the process of germination in some plant species.
- The tissue of rehydrated seeds activate the enzymes which help in
    - Hydrolysis or break down of storage food material (starch, proteins and fats)
    - Transfer of nutrients from cotyledons and endosperm to the growing points
    - Utilization of hydrolyzed products in the synthesis of new material
- Following the activation of enzymes, the embryo initiates growth in terms of cell division and elongation
- The hormones (GA, Auxins and Cytokinins) necessary for germination will be synthesized or activated.
- Growth of the root-shoot axis occurs at the expense of the storage tissue.

- The enlarging root-shoot axis exerts internal pressure on the seed coat and results in its rupture. In case of dicots the pressure developed between the cotyledons helps in rupturing of the seed coat and emergence of growing point.
- It is primary root that emerges first and supports in the establishment of seedling
- Finally, the seedling establishes and becomes autotrophic.

4.4 FACTORS AFFECTING GERMINATION:

The effects of the environment on seed germination is quite complex. A few of the major environmental factors are discussed here.

a. water : Water is the basic requirement for seed germination. It is essential for enzyme activation. Thus, permits breakdown, hydrolysis, translocation and use of reserve food material. How ever, extreme moisture may inhibit germination.

b. **Air(O_2 & CO_2) :**Most of the seeds germination well under normal concentration of gases (20% O_2, 0.03% CO_2 and 80% nitrogen gas). Among them adequate supply of O_2 must be available for oxidative process like breakdown of inhibitors.

Rice seeds can germinate even in the absence of O_2 (anaerobic conditions), although the seedlings are weak and abnormal. However, N_2 gas has no influence.

c. Temperature: The effect of temperature on germination can be expressed in terms of **cardinal temperatures** that are minimum, optimum and maximum temperature at which germination occurs. The optimum temperature may be defined as the temperature at which greatest percentage of germination is recorded with in the shortest period of time. Some of the seeds like wheat and barely can tolerate and germinate at minimum temperature of 3 – 5^o c. The optimum temperature for most seeds is between 15^oC (wheat) to 30^o C (rice). Maximum temperatures at which some species can tolerate and germinate is 35^0 C (groundnut), 40^0C (sorghum) and 50^0C (cantaloupe). [However, some species germinate at temperatures approaching the freezing point e.g. alpine seeds.]

The above examples provide a clue that seeds differ in their requirement of temperature for germination. The **low temperature pre – treatment** before germination, usually called **stratification**, and high temperature pre – treatment before germination, usually called **high temperature pre – treatment** are to change the macromolecular structure of the seeds which in its original form in some way prevents germination.

d. Light : The greater promotion of light on germination occurs in red region (660nm) followed by an inhibition zone in the far red region (730 nm). Such promotary and inhibitory effects of red and far-red light respectively on germination is mediated by phytochrome. It is referred to as biologically active in pfr form (far red absorbing form) and germination can proceed. Exposure to far – red light (730) reconverts phytochrome to the red absorbing form (pr) and germination is blocked.

e. Soil conditions : Saline conditions inhibit seed germination. High salt concentration prevents imbibition of water by the seed. The same osmotic effect is also observed when the fertilizers are placed in close contact with seeds.

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Lecture-5

GROWTH AND DEVELOPMENT

5.1 'Growth' - definition:

Growth and Development are the most fundamental and conspicuous characteristics of all living organisms. According to dictionary, growth is the advancement towards maturity and development is a gradual increase in size. The plant physiological definition of growth is 'an irreversible increase in mass, weight or volume of a living organism, organ or cell.

5.2 Growth Curve:

Typical growth pattern of an annual plant is represented in figure.3. This can be divided into three phases.

1. Lag period of growth:

   During this period the growth rate is quite slow because it is the initial stage of growth.

2. Log period of Growth

   During this period, the growth rate is maximum and reaches the top because at this stage the cell division and physiological processes are quite fast.

3. "Senescence period or steady state period :

   During this period the growth is almost complete and become static. Thus the growth rate becomes zero.

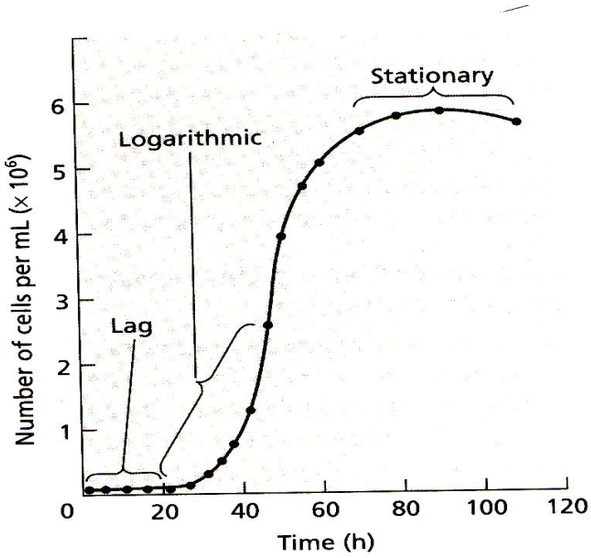


FIG.3 A TYPICAL 'S' SHAPED GROWTH CURVE

*Figure 3 is from reference 6

If the growth rate is plotted against time, an '**S**' shaped curve is obtained which is called **sigmoid curve** or **grand period curve**.

The growth curve described above is seen in most cases, although there is a considerable difference due to variations in plant species as well as environmental factors. It is also apparent that growth in all parts of a multi cellular plant is not uniform. In higher plants, it is restricted only to the **meristematic zones** which are found near the root and the shoot tips, in the vascular cambium and in certain parts of young leaves.

Growth pattern of annual crops:

Here the first phase relates to the seed germination and seedling growth. Seeds germinating below ground are dependent on stored material in the cotyledons until the seedling emerge in to light and start photosynthesis. Hence, initial increase in weight is negligible. **(LAG PHASE)**

The second phase of growth is characterized by a rapid and often linear increase in dry matter production and terminates with flowering(anthesis). **(LOG PHASE)** It is associated with tillering, stem elongation and leaf expansion in cereals. In case of **indeterminate crops** such as Cotton, Pigeon pea etc. it is characterized by formation of branches with large number of leaves.

Initiation of flower buds siginifies end of rapid growth phase **(grand period of growth)** and indicates onset of flowering.

The third phase of growth is marked by a reduction in growth rate until growth ceases at maturity. Assimilates stored in leaves and stems are translocated to partially sustain seed growth. At the end of this growth period, water is lost from aerial plant parts, photosynthesis stops and crop ripens **(STATIONARY PHASE AND DEATH)**.

5.3 TYPES OF GROWTH:

a) Determinate Organs :

Those organs that grow to certain size and then stop growing are called determinate organs. After their growth is completed they eventually senesce and die. Examples of such organs are leaves, flowers and fruits etc.

Determinate Growth:

If, reproductive growth starts only after completion of vegetative growth it is called as determinate growth habit.

Eg. Maize.

Indeterminate Organs:

Those organs which grow continuously with the activity of meristems are indeterminate organs. Examples are roots and vegetative stems of perennials. These structures always remain youthful, because of the meristematic activity.

Indeterminate Growth:

Here, vegetative and reproductive growth overlaps. This is shown in plants that have a capacity for both vegetative growth and flowering over an extended period.

Eg. Redgram, Soybean etc.,

5.4 Monocarpic and polycarpic species :

Monocorpic species flower only once and then die. Thus, in a sense mononcarpic species are determinate as for as the entire plant is concerned.

Ex.Rice, Maize,Sunflower,Sugarcane,sorghum etc.

Polycarpic species flower more than once in life cycle. Here, the vegetative and reproductive periods overlap each other. This is seen in most of the tree species.

Most monocrapic species are annuals. However, some of them are biennials and perennials also. Many varieties of bamboos may grow and live for over 50 years and then they flower and die. Thus bamboos are perennial but monocarpic. All polycarpic plants are perennials.

5.5 Development :

Growth leads to the development. Development is defined as ordered change or progress often (but not always) towards a higher, more ordered or more complex state. However, these two processes are often linked together and occur in sequence. Growth is a quantitative change in contrast to development which is more of a qualitative change.

5.5.1 INITIATION AND DEVELOPMENT OF VEGETATIVE STRUCTURES:

5.5.1.1 Root growth: Radicle is the embryonic root. During the seed germination and seedling formation, it grows to form primary root of the seedlings. A growing root usually has 4 distinct regions,

1. Root cap

2. Meristematic region

3. The region of cell elongation and

4. The region of differentiation and maturation.

The root cap protects the root tip. The meristematic region in young root is situated just below the root tip. The cells in this region are responsible for growth in the root. The meristematic region consists of numerous small, compactly arranged thin walled cells almost completely filled with cytoplasm. They have very small vacuoles and comparatively large nuclei. Inter cellular spaces are absent. Only a few cells in the meristem may actually be involved in the longitudinal growth of the root.

F.A.L. Clowes and B.E. Jupiner (1968) demonstrated that there is a **quiescent center** in the meristematic region, where no cell division takes place. This center is located just above the root tip. It is surrounded by a group of actively dividing cells, which give rise to the column of cells forming roots.

The region of cell elongation is made up of column of newly derived cells. It is the elongation of these cells, which causes the root tip to project forward and push through. Most cells in this region elongate at least 15 folds and increase in diameter which results in the development of considerable pressure by the elongation of root.

The region of differentiation and maturation:

The cells in the region of differentiation and maturation differentiate into various tissues, characteristic to the mature root; the epidermis, cortex and stele.

In roots xylem and phloem differentiate only acropetally and as continuation of the older xylem and phloem in the more basal part of the root.

During differentiation most cells increase in size and vacuolation.

5.5.1.2 Stem growth : The life of stem starts as a plumule. It grows to form the shoot of the seedling. The longitudinal growth of stem and formation of various organs like branches, leaves, flowers is the function of stem meristem.

Tunica Corpus Theory:

To explain the cellular organizations of stem meristem, **A.Schmidt** (1924) first proposed a **tunica corpus theory**. Accordingly , most apical meristems contain two zones, an outer tunica and an inner Corpus. Tunica consists of one to several layers of cells at the surface of the merisem while corpus cells are beneath the tunica layer. The cells in tunica divide by anticlinal division i.e in a plane perpendicular to the surface of the stem, whereas the corpus cells divide in many different ways.

The formation of branches leaves or outer appendages on the stem are initiated in the formation of a primordium or out growth at the surface of the meristem, just below the tip. In the formation of aerial organs both tunica as well as corpus layers are involved. The tunica normally forms the epidermis of the organ derived from the

meristem, while corpus cells produce majority of the internal tissues of the new organ.

Auxins normally promote the elongation of stem. They induce the elongation of cells. Gibberellins also promote stem elongation and they do this by promoting cell division as well as cell elongation.

5.5.1.3 Leaf initiation and Growth: Elevations appear on the periphery of the meristem in a regular pattern. Leaf primordia appear as dome shaped on the periphery of the stem. They appear at nodal positions of the stem, which have an **intercalary meristem** when the leaves are to be produced in pairs; each pair usually appears to right angle to the preceding pair, the two leaves in a pair generally opposite to each other.

The growth of individual leaf also follows the typical sigmoidal pattern, just like the growth of the entire plant. In most plants, the shape and form of leaves are fixed and little variation found among them.

However, many plants have leaves of different shape. The phenomenon is termed as heterophylly, which is quite common in aquatic plants.

5.5.2 INITATION AND DEVELOPMENT OF REPRODUCTIVE STRUSCTURES

5.5.2.1 Initiation and Development of Flower:

Once the biochemical requirements for evocation of flowering are completed and the meristem has reached the point of no return, it develops either into an inflorescence (a cluster of flowers) or solitary flowers. In most plants, the pattern of flower initiation and development is almost similar. As an example of flower initiation in *Capsicum annum*(Green pepper) the first microscopically visible change in the shoot apex is the change in its shape. The apex almost becomes flat from conical, due to the inhibition of growth in the central portion of the meristem. Some protuberances develop from this meristem in a whorled manner. Floral parts (sepals, petals etc) are formed due to the development of the protuberances. The outermost whorl of the protuberances forms the sepal and next to it forms petals and so on.

Most plants produce bisexual flowers containing functional male (stamens) and female (pistils) parts. Other species contain staminate (male) and pistillate (female) flowers only on different individual plants.

Auxins and Ethylene stimulates the formation of female flowers, where as gibberellins increase the ratio of male to female flowers in the cucumber.

Initially, the floral parts are tightly enclosed with in the outer most part, the sepals, constituting a floral bud. Subsequently expansion of the flower bud in to an open flower occurs. The cause of the flower opening is usually due to the differential growth of the inner and outer sides of the sepals and petals.

5.5.2.2 Fruit and Seed Development:

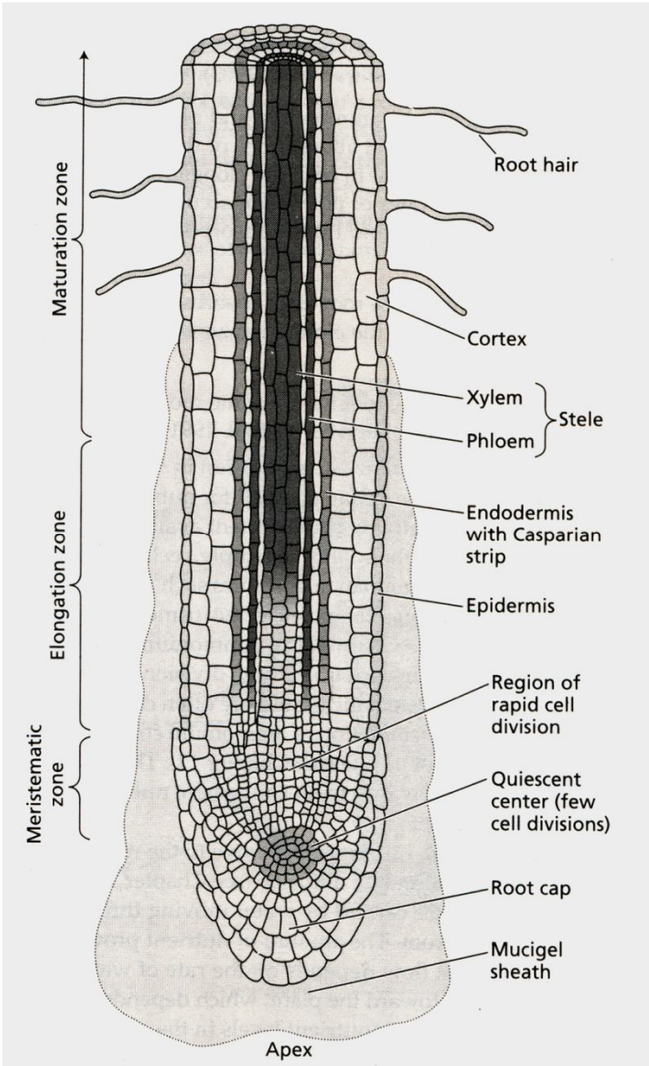
The first stage in fruit and seed development is rapid cell division without much enlargement due to cytokinin production by the endosperm which is growing at this stage. Various tissues of the parent plant *viz*, the ovary, receptacle and sometimes parts of the floral tube may be involved in the formation of fruits.

Following the cell division stage cell enlargement phase of growth proceeds and this is by auxins produced in the seeds. If the seeds are removed from a developing fruit, development stops, however it can be restarted again by the application of auxins.

It was observed that fruit development in cucumber is dependent on auxins which originate from the ovule, while some fruits respond rather to gibberellins than to auxin treatment.

At this stage in the development of fruits the concentration of organic acids and sugars begin to increase followed by decrease in osmotic potential. This is related to the increasing absorption of water and growth by enlargement of cells.

ANNEXURE



*Figures are from reference 6

Lecture-6

GROWTH AND DEVELOPMENT

6.1 MEASUREMENT OF GROWTH: Growth can be measured by a variety of parameters as follows

A. Fresh Weight

Determination of Fresh weight is an easy and convenient method of measuring growth. For measuring fresh weight, the entire plant is harvested, cleaned for dirt particles if any and then weighed.

B. Dry Weight

The dry weight of the plant organs is usually obtained by drying the materials for 21 to 48 h at 70 to 80^{o}C and then weighing it. The measurements of dry weight may give a more valid and meaningful estimation of growth than fresh weight. However, in measuring the growth of dark grown seedling it is desirable to take fresh weight.

C. Length

Measurement of length is a suitable indication of growth for those organs which grow in one direction with almost uniform diameter such as roots and shoots. The length can be measured by a scale. The advantage of measuring length is that it can be done on the same organ over a period of time without destroying it.

D. Area

It is used for measuring growth of plant organs like leaf. The area can be measured by a graph paper or by a suitable mechanical device. Nowadays modern laboratories use a photoelectric device (digital leaf area meter) which reads leaf area directly as the individual leaves are fed into it.

6.2 GROWTH ANALYSIS:

Growth analysis is a mathematical expression of environmental effects on growth and development of crop plants. This is a useful tool in studying the complex interactions between the plant growth and the environment. Growth analysis in crop plants was first studied by British Scientists (Blackman 1919, Briggs, Kidd and west 1920, William 1964, Watson 1952 and Blackman, (1968). This analysis depends mainly on primary values (Dry weights) and they can be easily obtained without great demand on modern laboratory equipment.

The basic principle that underlie in growth analysis depends on two values (1) total dry weight of whole plant material per unit area of ground (w) and (2) the total leaf area of the plant per unit area of ground (A).

The total dry weight (w) is usually measured as the dry weight of various plant parts *Viz*, leaves, stems and reproductive structures. The measure of leaf area (A) includes the area of other organs *viz,* stem petioles, flower bracts, awns and

pods that contain chlorophyll and contribute substantially to the over all photosynthesis of the plants

According to the purpose of the data, leaf area and dry weights of component plant parts have to be collected at weekly, fortnightly or monthly intervals. This data are to be used to calculate various indices and characteristics that describe the growth of plants and of their parts grown in different environments and the relationship between assimilatory apparatus and dry matter production. These indices and characteristics are together called as **growth parameters.** Some of the parameters that are usually calculated in growth analysis are crop growth rate (CGR), relative growth rate (RGR), net assimilation rate (NAR), Leaf area ratio (LAR), Leaf weight ratio (LWR). Specific Leaf Area (SLA), Leaf area index (LAI) and Leaf area duration (LAD). Accuracy in calculations of these parameters and their correct interpretation are essential aspect in growth analysis.

6.2.1 Advantages of growth analysis

a) We can study the growth of the population or plant community in a precise way with the availability of raw data on different growth parameters.
b) These studies involve an assessment of the primary production of vegetation in the field i.e. at the ecosystem level (at crop level) of organization.
c) The primary production plays an important role in the energetics of the whole ecosystem.
d) The studies also provide precise information on the nature of the plant and environment interaction in a particular habitat.
e) It provides accurate measurements of whole plant growth performance in an integrated manner at different intervals of time.

6.2.2 Drawbacks of Growth Analysis

In classical growth analysis sampling for primary values consist of harvesting (destructively) representative sets of plants or plots and it is impossible to follow the same plants or plots through out whole experiment.

6.3 Growth Characteristics – Definition and Mathematical Formulae

The following data are required to calculate different growth parameters in order to express the instantaneous values and mean values over a time interval. In the following discussion W, W_L, W_S and W_R are used to represent the dry weights of total plant(w), dry leaves(w_L), stem(W_S) and roots(W_R) respectively. Whereas A is the leaf area and P is the land area.

6.3.1 Crop Growth Rate (CGR):C

D.J. Watson coined the term Crop growth rate. It is defined as the increase of dry matter in grams per unit area per unit time. The mean CGR over an interval of time T_1 and T_2 is usually calculated as show in the following formula

$$CGR = \frac{I}{P} \times \frac{W_2 - W_1}{T_2 - T_1} \quad (g\ m^{-2}\ day^{-1})$$

Where CGR is the mean crop growth rate, W_1 and W_2 are the dry weights at two sampling times T_1 and T_2 respectively.

6.3.2 Relative Growth Rate (RGR):R

The term RGR was coined by Blackman. It is defined as the rate of increase in dry matter per unit of dry matter already present. This is also referred as **Efficiency index** since the rate of growth is expressed as the rate of interest on the capital. It provides a valuable overall index of plant growth. The mean relative growth rate over a time interval is given below.

$$RGR = \frac{Log_e W_2 - Log_e W_1}{T_2 - T_1} \quad (g\ g^{-1}\ day^{-1})$$

6.3.3 Net Assimilation Rate (NAR):E:

The NAR is a measure of the amount of photosynthetic product going into plant material i.e. it is the estimate of net photosynthetic carbon assimilated by photosynthesis minus the carbon lost by respiration. The NAR can be determined by measuring plant dry weight and leaf area periodically during growth and is commonly reported as grams of dry weight increase per square centimeter of leaf surface per week. This is also called as **Unit leaf rate** because the assimilatory area includes only the active leaf area in measuring the rate of dry matter production.

The mean NAR over a time interval from T_1 to T_2 is given by

$$NAR = \frac{W_2 - W_1}{T_2 - T_1} \times \frac{Log\ e\ A_2 - Log\ e\ A_1}{A_2 - A_1} \quad (g\ cm^{-2}\ wk^{-1})$$

6.3.4 Leaf Area Ratio (LAR)

The LAR is a measure of the proportion of the plant which is engaged in photosynthetic process. It gives the relative size of the assimilatory apparatus. It is also called as capacity factor. It is defined as the ratio between leaf area in square centimeters and total plant dry weight. It represents **leafiness character** of crop plants on area basis.

$$LAR = \frac{A}{W} \quad (cm^2 g^{-1})$$

6.3.5 Leaf Weight Ratio (LWR)

It is one of the components of LAR and is defined as the ratio between grams of dry matter in leaves and total dry matter in plants (g). Since the numerator and denominator are on dry weight basis LWR is dimensionless. It is **the index of leafiness** of the plant on weight basis.

$$LWR = \frac{W_L}{W}$$

6.3.6 Specific Leaf Area (SLA)

It is another component of LAR and defined as the ratio between leaf area in cm^2 and total leaf dry weight in grams. This is used as a measure of **leaf density.** The mean SLA can be calculated as

$$SLA = \frac{A}{W_L} \quad (cm^2 g^{-1})$$

6.3.7 Specific Leaf Weight (SLW)

The reversal of SLA is called as SLW. It is defined as the ratio between total leaf dry weight n gms and leaf area in cm^2. It indicates the **relative thickness** of the leaf of different genotypes.

$$SLW = \frac{W_L}{A} \quad (g\ cm^{-2})$$

6.3.8 Leaf area index (LAI):

D.J. Watson coined this term. It is defined as the functional leaf area over unit land area. It represents the leafiness in relation to land area. At an instant time (T) the LAI can be calculated as

$$LAI = A/P \text{ unit less}$$

For maximum production of dry matter of most crops, LAI of 4-6 is usually necessary. The leaf area index at which the maximum CGR is recorded is called as 'optimum leaf area index'.

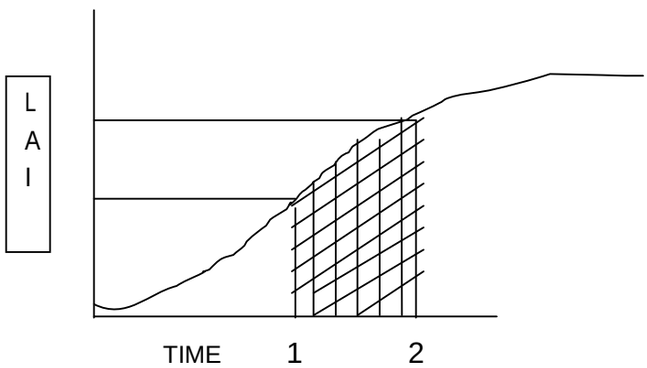
6.3.9 Leaf Area Duration (LAD): d: It is usually expressed as a measure of leaf area integrated over a time period. Some takes into account both the magnitude of leaf area and its persistence in time;

it represents the leafiness of the crop growing period. Thus the unit of measurement of LAD may be in days or weeks or months.

$$\text{LAD (Leaf area basis)} = \frac{LA_1 + LA_2(T_2 - T_1)}{2}$$

$$\text{LAD (LAI basis)} = \frac{LA_1 + LA_2\,(T_2 - T_1)}{2}$$

This is expressed as $cm^2\ d^{-1}$



Leaf area duration (Shaded area)

For how many days/weeks functional leaf area is present on the plant can be estimated by this LAD.

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Lecture-7

CROP WATER RELATIONS

Almost every plant process is affected directly or indirectly by the water supply. Decreasing water content is accompanied by loss of turgor and wilting, cessation of cell enlargement, closure of stomata, reduction in photosynthesis and interference with many basic metabolic processes. Eventually, continued dehydration causes disorganization of the protoplasm and death of most organisms.

7.1 PHYSIOLOGICAL IMPORTANCE OF WATER:

The importance of water can be summarized under the following general headings.

a) Constituent of Protoplasm

Water is important quantitatively and qualitatively, constituting 80 to 90 percent of the fresh weight of most herbaceous plant parts and over 50 percent of the fresh weight of woody plants. Water is important as a part of the protoplasm as the protein molecules which constitute the protoplasm framework, changes in structure if the water content is dropped below certain level. This ultimately leads to death of plants. It is true that a few plants and organs can be dehydrated to the air-dry condition or even to the oven-dry condition as in the case of some kinds of seeds and spores, without loss of viability, but a marked decrease in physiological activity always accompanies decrease in water content.

b) Solvent

A second essential function of water in plants is as the solvent in which gases, minerals and other solutes enter plant cell and move from cell to cell and organ to organ. The permeability of most cell walls and membranes to water in a continuous liquid phase extending throughout the plant in which translocation of solutes of all kinds occurs.

c) Reagent

Water is a reactant or reagent in many important processes, including photosynthesis and hydrolytic process such as the hydrolysis of starch to sugar. It is also essential for fixation of carbon dioxide or nitrate.

d) Maintenance of Turgidity

Another essential role of water is the maintenance of the turgidity which is essential for cell enlargement and growth, and for maintaining the form of herbaceous plants. Turgor is also important in the opening of stomata and the movements of leaves, flower petals and various specialized plant structures. Inadequate water to maintain turgor results in an immediate reduction of vegetation growth.

7.2 WATER POTENTIAL AND ITS COMPONENTS:

Water potential or chemical potential of water is a quantitative expression of the free energy associated with water.

Water potential is symbolized by the Greek letter Ψ (psi) and is defined relative to the water potential of pure water, which is zero. Hence the value of Ψ is always negative. The units of water potential are mega Pascal (MPa). It is a relative quantity and depends on concentration, pressure and gravity at the same temperature.

Water potential as the sum of component potentials which may be written as

Ψ = Ψs + Ψm + Ψp + Ψg

Where Ψs = Solute or osmotic potential (symbol π)

Ψm = Matric potential (Symbol T)

Ψp = Pressure potential (Symbol P)

Ψg = Gravitational potential (Symbol G)

Osmotic potential

The osmotic potential, Ψs (or π) is the component produced by solutes dissolved in the cell sap, chiefly vacuolar sap.

Matric Potential

The matric potential, Ψm (or T) refers to water held in micro capillaries or bound on surfaces of the cell walls and other cell components.

Pressure potential

The pressure potential Ψp (or P) is the turgor pressure produced by diffusion of water into protoplasts enclosed in walls which resist expansion. In the xylem of transpiring plants Ψp is usually negative and in guttating plants it is positive as a result of root pressure.

Gravitational Potential

The effect of gravity, Ψg (or G) is a term of negligible importance within root or a leaf but becomes important in comparing potentials in leaves at different heights on trees and in soils.

Upward movement of water in a tree trunk must overcome a gravitational force of 0.01 Mpa/m and gravity causes drainage of water downward in soil.

The volume of matric water is very small compared the volume of vacuolar water in parenchyma, therefore Ψm has a negligible effect on the total water potential Ψ. However, in developing seeds or thick walled cells where the vacuolar

water constitutes a small fraction of the total water, matric potential can control the cell water potential.

Thus for herbaceous plants and annual field crops of a short vertical height (less than 10 m) the values of the matric potential and gravitational potential are small and are commonly omitted. Thus

$$\Psi = \Psi s + \Psi p \text{ or } P\text{-}\pi$$

Water always moves from less negative water potential to more negative water potential.(Figure.4)

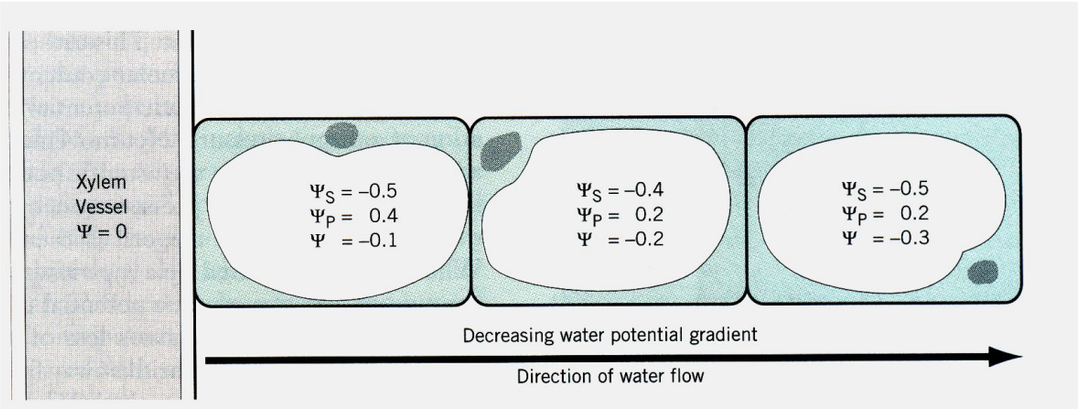


*Figure.4 is from reference 5

7.3 IMPORTANCE OF WATER POTENTIAL:

Water potential is a diagnostic tool that enables the plant scientist to assign a precise value to the water status in plant cells and tissues.

The lower the water potential in a plant cell or tissue, the greater is its ability to absorb water. Conversely, the higher the water potential, the greater is the ability of the tissue to supply water to other more desiccated cells and tissues.

Thus water potential is used to measure water deficit and water stress in plant cells and tissues.

As a general rule, leaves of most plants rooted in well watered soils are likely to have water potentials between about -2 and -8 bars. With decreasing soil moisture supply, leaf water potential will become more negative than -8 bars and leaf growth rates will decline. Most plant tissues will cease growth completely (i.e., will not enlarge) when water potential drops to about -15bars.

7.4 UPTAKE OF WATER

The way in which water is entered in to the root hair and the precise mechanism of water absorption is has been explained by two different approaches.

(a)Active uptake:

Water is absorbed as a result of activities in the root itself and does not concern with any process in shoot.

(b)Passive uptake of water:

The governing force of water absorption originates in the cells of transpiring shoots rather than in root itself.

Although the absorption of water by roots is believed to be a passive, pressure driven process, it is nonetheless dependent on respiration. Respiratory inhibitors (such as cyanide),anaerobic conditions (waterlogged condition) decrease in the hydraulic conductance of most roots .These are some supporting points for active absorption of water. How ever the exact role of respiration and active uptake is not clear.

Barring few exceptions, it is now believed that uptake of water is a Passive process. Tension or negative pressure originating at the actively transpiring leaf surface creates a pulling force for water movement in xylem.(Cohesion-tension theory of Dixon and Jolly)

The movement of water inside the plant is driven by a reduction in free energy, and water may move by diffusion, by bulk flow or by a combination of these fundamental transport mechanisms. Water diffuses because molecules are in a constant thermal agitation, which tends to even out concentration differences. Water moves by bulk flow in response to a pressure difference, when ever there is a suitable path way for bulk movement of water. Thus, water potential difference (i.e solute potential and pressure potential) across the cells starting from root hairs to xylem plays an important role in uptake and transport of water.

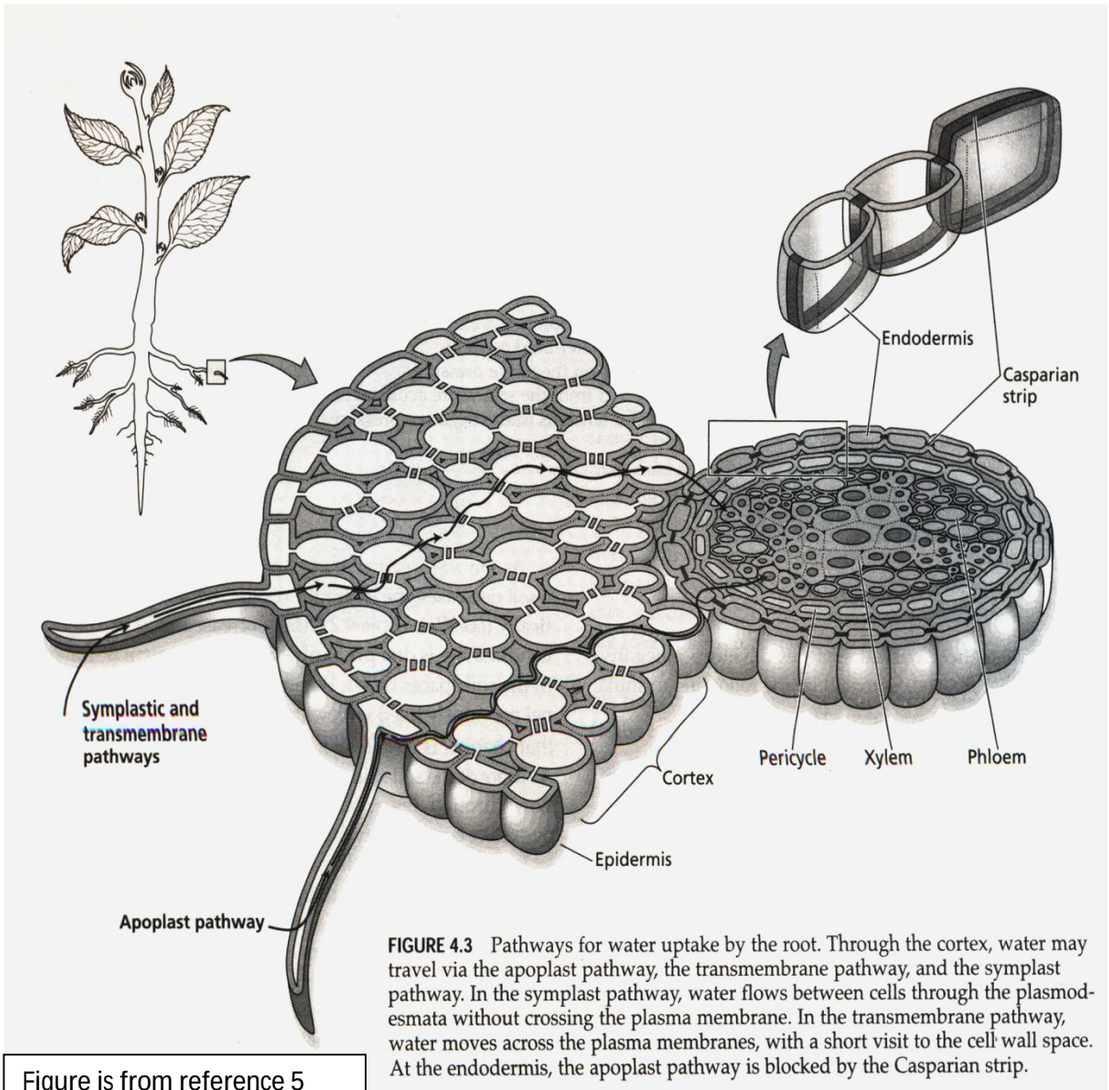


FIGURE 4.3 Pathways for water uptake by the root. Through the cortex, water may travel via the apoplast pathway, the transmembrane pathway, and the symplast pathway. In the symplast pathway, water flows between cells through the plasmodesmata without crossing the plasma membrane. In the transmembrane pathway, water moves across the plasma membranes, with a short visit to the cell wall space. At the endodermis, the apoplast pathway is blocked by the Casparian strip.

Figure is from reference 5

7.5 METHODS OF MEASURING WATER STATUS IN PLANTS:

There are two general ways to describe the water status or internal water balance of plant and plant tissue. The first one is based on the energy associated with water in the plant tissue. Water potential is considered by most plant physiologists to be the most useful and significant way to describe the water status of plant tissues. In terms of water potential, water deficit exists in a tissue when ever its water potential is less i.e., more negative than zero mega Pascal (Mpa). The water potential is measured by (1) liquid immersion method (dye method) (2)vapor equilibration method (Thermocouple Psychrometer) and (3)pressure chamber method.

7.5.1 Liquid immersion method or dye method or Chardakov's Falling Drop Method

Tow graded series of sucrose solutions (ranging from 0.15 to 0.50 molal in increments of 0.5molality) are placed in test tubes set up in duplicate. Homogeneous plant tissue is placed into each test tube of one of the series (test series). Only a drop of methylene blue is mixed into each solution of the second series (control series) Plant tissue is not added to the control series and the dye does not appreciably change the osmotic potentials.

After the tissue is incubated for 15 to 30 minutes, It is removed from each tube. The actual time of incubation can be just long enough for osmosis to proceed and change the concentration of each solution in the test series; the attainment of equilibrium is not necessary. After the tissue is removed, a small drop of the respective control series solutions is introduced below the surface of its corresponding test solution. If the drop rises in the test solution, it means that the drop is lighter and that the tissue incubation solution is more concentrated – an indication that water from the solution entered the tissue. Conversely, if the drop falls, it means that the test solution is lighter-an indication that water has left the tissue and diluted the solution. In this latter instance, the water potential of the solution initially is more negative than that of the tissue. Accordingly, if the density of the drop from the methylene blue solution is the same as that of the test solution, the drop will diffuse into the solution uniformly. At this point (called the null point), the water potential of the tissue and solution is equal.

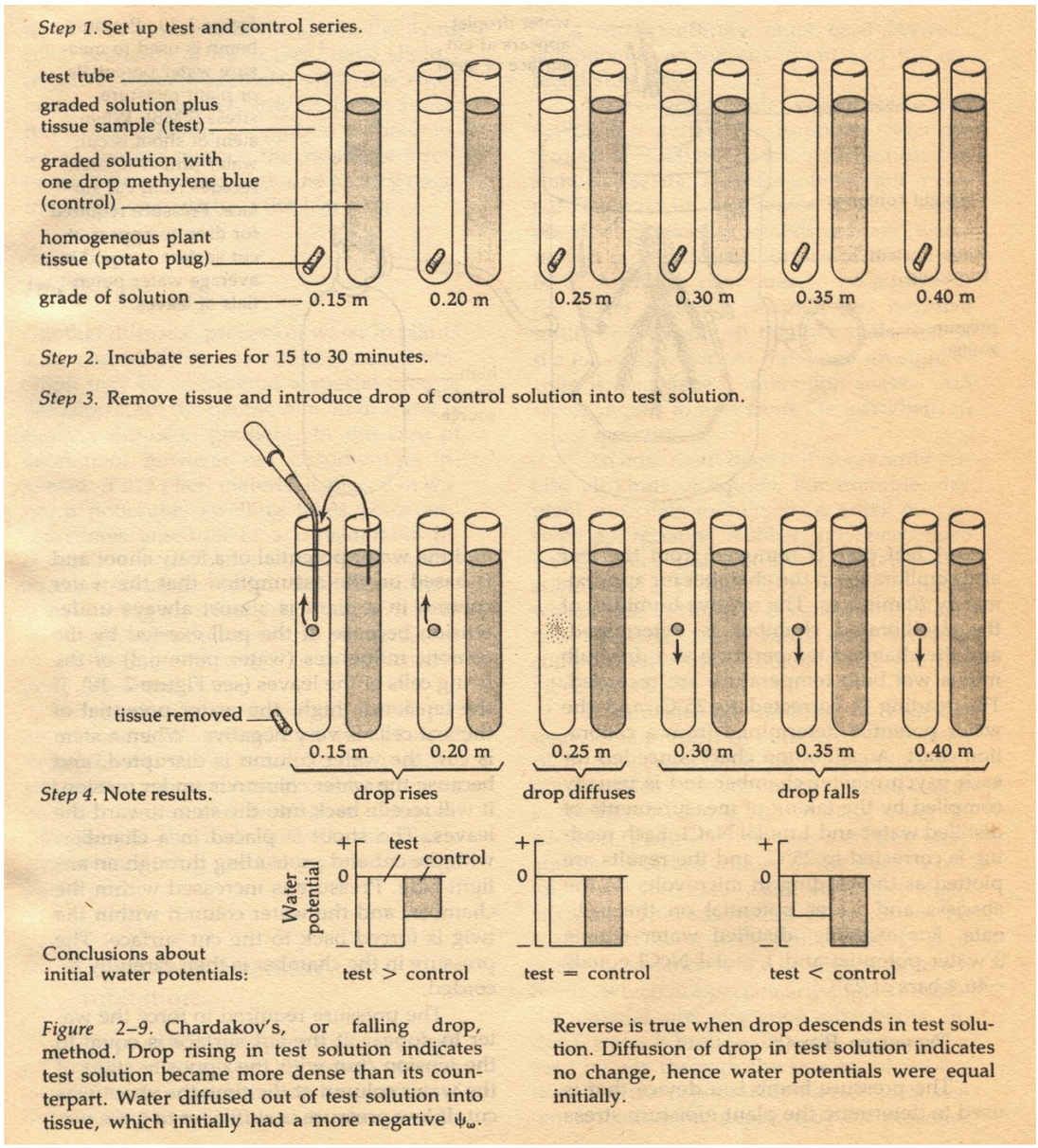
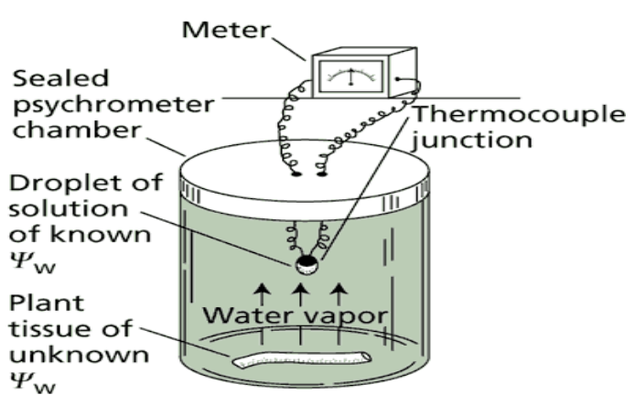


Figure 2–9. Chardakov's, or falling drop, method. Drop rising in test solution indicates test solution became more dense than its counterpart. Water diffused out of test solution into tissue, which initially had a more negative ψ_ω. Reverse is true when drop descends in test solution. Diffusion of drop in test solution indicates no change, hence water potentials were equal initially.

7.5.2 Vapour equilibration (Thermocouple Psychrometer) Method

Psychrometry (the prefix "psychro-" comes from the Greek word *psychein*, "to cool") is based on the fact that the vapor pressure of water is lowered as its water potential is reduced. Psychrometers measure the water vapor pressure of a solution or plant sample, on the basis of the principle that evaporation of water from a surface cools the surface.



Investigators make a measurement by placing a piece of tissue sealed inside a small chamber that contains a temperature sensor (in this case, a thermocouple) in contact with a small droplet of a standard solution of known solute concentration (known Ψ_s and thus known Ψ_w). If the tissue has a lower water potential than that of the droplet, water evaporates from the droplet, diffuses through the air, and is absorbed by the tissue. This slight evaporation of water cools the drop. The larger the difference in water potential between the tissue and the droplet, the higher the rate of water transfer and hence the cooler the droplet. If the standard solution has a lower water potential than that of the sample to be measured, water will diffuse from the tissue to the droplet, causing warming of the droplet. Measuring the change in temperature of the droplet for several solutions of known Ψ_w makes it possible to calculate the water potential of a solution for which the net movement of water between the droplet and the tissue would be zero signifying that the droplet and the tissue have the same water potential.

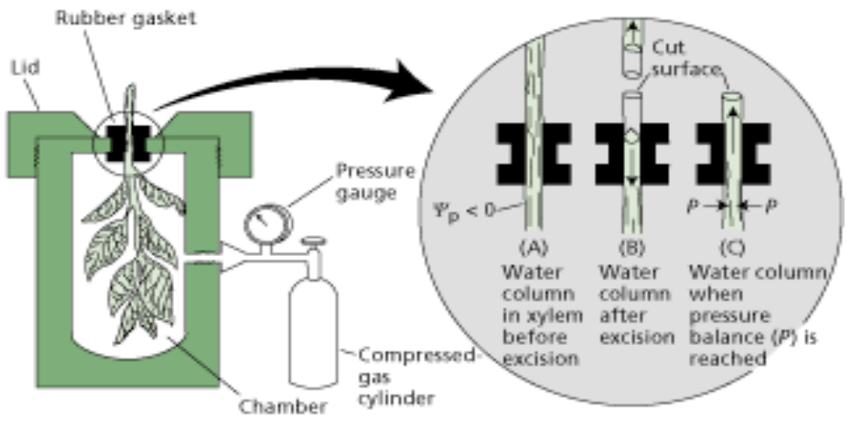
Psychrometers can be used to measure the water potentials of both excised and intact plant tissue. Moreover, the method can be used to measure the Ψ_s of solutions. This can be particularly useful with plant tissues.

A major difficulty with this approach is the extreme sensitivity of the measurement to temperature fluctuations. For example, a change in temperature of 0.01°C corresponds to a change in water potential of about 0.1 MPa. Thus, psychrometers must be operated under constant temperature conditions. For this reason, the method is used primarily in laboratory settings.

7.5.3 Pressure chamber method

A relatively quick method for estimating the water potential of large pieces of tissues, such as leaves and small shoots, is by use of the **pressure chamber**. This method was popularized by **P. Scholander** and coworkers.The pressure bomb is a device that is used to determine the plant moisture stress and the water potential of a leafy shoot and in based on the assumption that the water column in a plant is almost always under tension because of the pull exerted by the osmotic influences (water potential) of the living cells of the leaves. If the tension is high, the water potential of the leaf cells is very negative. When a stem is cut, the water column (in xylem) is disrupted and because the water column is under tension, it will recede back into the stem toward the leaves. The shoot is placed in a chamber, with the cut end protruding through an airtight hole. Pressure is increased within the chamber and the water column with in the twig are forced back to the cut surface. The pressure in the chamber is then carefully recorded.

The pressure required to force the water to appear at the cut surface is equal to the tension (but with the opposite sign) of the water column at the time the shoot was cut. If low pressure is sufficient to force water to the cut surface of the shoot, the shoot is under relatively low moisture stress. But if high pressure is required to force to the cut surface the moisture stress (tension) is relatively high due to very negative water potential of the leaf cells.



7.5.2 The second way to describe water status is to measure the quantity of water in a tissue i.e. its water content and to express it in relation to a selected references. Three of these methods are

-fresh weight method
- dry weight method and
- relative water content (RWC) method.

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Lecture-8

CROP WATER RELATIONS

8.1 TRANSPIRATION:

The loss of water from aerial parts of plants in the form of vapor is known as transpiration. The loose arrangement of the living thin walled mesophyll cells, which results in an abundance of inter cellular space provides an ideal condition for the evaporation of water from internal leaf surface. Part of the epidermal surface of the leaf is made up of a great number of microscopic pores called stomata. Water vapor collected in the intercellular spaces of leaf mesophyll diffuse into the atmosphere through the open stomata. This form of transpiration is termed as **stomatal transpiration**.

In addition to stomatal transpiration, water is lost as vapor directly from leaf surfaces and through lenticels (small opening in the corky tissue covering stems and twigs). The former is called cuticular transpiration and the later lenticular transpiration.

Stomatal Transpiration

The stomatal transpiration accounts to 80 to 98% of the total transpiration loss from plants (tree to herbaceous plants). Under very dry conditions, stomata are closed and water loss occurs through the cuticle and lenticels.

Cuticular Transpiration

The cuticular transpiration accounts to 2 to 20% of the total transpiration loss from plant (xerophytes to mesophytes). The cuticle although retards water loss, is some what permeable to water vapor. In plants with thick cuticles, this form of transpiration is insignificant.

Lenticular Transpiration

The lenticular transpiration amount to 0.1 to 1% of the total transpiration loss from plants which is insignificant when compared to stomatal transpiration. However, lenticular transpiration may cause some desiccation in those trees that shed their leaves at the on set of the winter. During cold winter water absorption by roots is at a minimum, thus the importance of lenticular transpiration is increased.

8.2 SIGNIFICANCE OF TRANSPIRATION

Transpiration is advantageous because.

- It creates suction force and help in the ascent of sap.
- It helps in the absorption of water and minerals by roots.
- It helps in evaporating excess amount of water from moist soil.
- It plays a role in translocation of food from one part of the plant to the other.
- It brings opening and closure of stomata which indirectly influences the gaseous exchange for the processes of photosynthesis and respiration.

- It helps in dissipating the excess energy absorbed from the sun, which will otherwise raise the leaf temperature.
- It maintains suitable temperature of leaves by imparting a cooling effect.

Transpiration is regarded as an unavoidable (necessary) evil. It is unavoidable because leaf structure (stomata) favorable for uptake of Co_2 and O_2 necessary for photosynthesis and respiration is also favorable for the loss of water through transpiration.

Transpiration is an 'evil' because often it causes injury by dehydration due to heavy transpiration loss when the atmospheric conditions are aggressive such as high light intensity, hot winds, depleted soil moisture and poor water retentive capacity of soil.

8.4 STRUCTURE OF STOMATAL COMPLEX IN MONOCOT AND DICOT SPECIES

The appearance of the guard cells differs characteristically from the surrounding epidermal cells. The guard cells of some plant species, particularly grasses (monocots), are **dumbbell shaped** and are associated with epidermal cells that also differ in appearance from the rest of the epidermal cells. These epidermal cells are called subsidiary cells or accessory cells. In case of dicots, the guard cells are generally **bean shaped**. (Fig.5)

One other distinguishing feature of guard cell is the presence of chloroplasts. Epidermal cells do not possess chloroplasts.

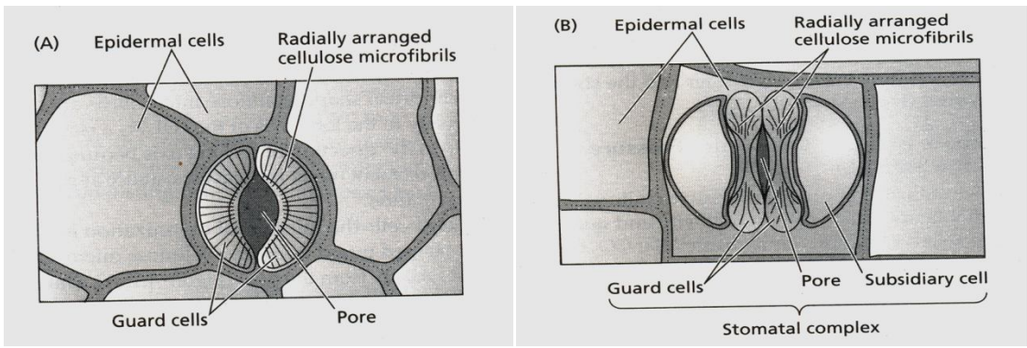

*Figure.5 is from reference 6

8.4.1 INVOLVEMENT OF STOMATA IN TRANSPIRATION :

The stomatal movement is generally understood as a direct response to increase or decrease in the osmotic potential that result from osmotic changes that cause water to move in or out of the guard cells. If water moves in, the cells expand (become **turgid**); if water moves out, they go **flaccid**. When the guard cells are turgid, the stoma is open; when the guard cells are flaccid, the stoma is closed. To effect this movement of water, an exchange must takes place between the guard

cells and the surrounding mesophyll and epidermal cells. The development of a **more negative osmotic potential** in the guard cells would cause of water potential gradient to develop between the guard cells and their neighboring cells. Water would diffuse in to the guard cell, causing them to become more turgid. The development of a **less negative osmotic potential** in the guard cells would of course, cause a water potential gradient to develop in the opposite direction, and water would flow out of the guard cells into the neighboring cells. (see the diagram in the annexure)

8.5 TRANSPIRATION IN RELATION TO PRODUCTIVITY:

The importance of water use efficiency (WUE) in influencing grain yield under water limited conditions can be explained by the following model give by passioura.

Grain Yield = T x TE x HI

Where T = Total transpiration by the crop canopy

TE = Transpiration Efficiency or WUE

HI = Harvest Index (Economic Fraction of Dry matter)

This relationship provides an analytical tool to select the genotype with high levels of T and TE.

8.6 WATER USE EFFICIENCY:

The water use efficiency (WUE) of field crops is defined as follows:

'It is the amount of dry matter produced per unit amount of water transpired'

$$WUE = \frac{\text{Dry matter production (DM)}}{\text{Evapotranspiration}}$$

This is expressed as g DM kg^{-1} water WUE measurements can be made on plants in containers, on individual plants, and on crop communities. They can be used for economic yield as well as total dry matter calculations.

A related term, **water requirement** is the reciprocal of WUE. Water requirements is usually expressed in weights of equal magnitude, such as g water $(g\ DM)^{-1}$

8.7 Water use efficiency in C_3 & C_4 plants.

Field data for WUE, when regrouped into C_3 and C_4 species, illustrate a two fold increase for C_4 species when calculated for either grasses or dicots

Water use Efficiency (g DM $(kgH_2O)^{-1}$) for C_4 and C_3 species.

Species	Grasses	Dicots
C_3	1.49	1.59
C_4	3.14	3.44

Large differences in WUE occur when species are categorized by Co_2 fixation pathway. It is now accepted that the WUE of C_4 species is generally higher than C_3 species. The higher WUE of C4 species is a result of higher photosynthetic rate under high light and temperature and lower transpiration rates under low light.

The WUE values for both C3 and C4 species are low compared with CAM plants. One CAM species, pineapple (*Ananas comosis*), has shown a WUE of 20g, DM kg^{-1} water. Use of crop species with CAM is limited because the Co_2 fixation and overall productivity of CAM plants is low (CAM is only a survival mechanism but not a productive mechanism).

8.8 WUE of major field crops :

Crop	Co2 Fixation pathway	water requirement (gH_2O, gDM^{-1})	water Use Efficiency (g DM kg^{-1} H_2O)
Maize	C_4	388	2.58
Sorghum	C_4	402	2.49
Potato	C_3	532	1.88
Sugar beet	C_3	606	1.65
Wheat	C_3	613	1.63
Soybean	C_3	704	1.42
Alfalfa	C_3	993	1.01

8.9 FACTORS INFLUENCING THE WATER USE EFFICIENCY:

1. Climatic factors

WUE has almost an inverse relationship with Relative humidity (R.H), lower R.H. increases evapotranspiration (ET) without a corresponding increase in crop yield. On the other hand, factors such as sunlight and temperature that affect ET, rate of photosynthesis and dry matter production will either decrease or increase WUE.

2. Agronomic practices and crop management

Early sown crops will escape the moisture stress while the delayed sowing favors heavy weed growth which creates severe competition for water, light, nutrients etc. the crop should have an optimum crop canopy (4-6 LAI for most crops) for proper light interception. Plant Population and plant architecture influence WUE by influencing the interception and utilization of solar energy. Depth of sowing also influences water availability and there by seedling emergence, vigor and final yield.

3. Antitranspirants

WUE can also be improved by using antitranspirants which reduces transpiration. These antitranspirant may influence stomatal closure (Phenyl mercuric acetate, ABA, CCC Salicylic acid etc) or form a film (Hexadecanol, cetyl alcohol etc.) on the leaf surface or increase plant reflectivity (eg. Kaolinite) and reduce leaf temperatures.

4. Use of mulches

Mulches are beneficial in conserving or economizing water use by plant ranging from 10-50%. It depends upon the crop in which it is used, rainfall, wind velocity and temperature of both air and soil. Organic mulches (straw, rice dust, saw dust etc.) light colored and light reflecting mulches reduce soil temperature. But black colored mulches such as black, grey, transparent polythene sheets and petroleum products increase temperature up to 5-8^{0}C. Plastic mulches are costly and suitable to areas where soil temperatures are low and unfavorable to crop growth.

5. Use of shelter belts

Shelter belts decrease the damaging effects of winds on crops and modify the micro climate. Higher humidity and lower vapor pressure deficit prevail in shelter belt that reduces heat. This will help ultimately for higher WUE. Increased yields due to shelter belts are reported in case of cotton, onion, sweet potato, tomato and wheat.

6. Method and quantities of water application.

Frequent light irrigations keep the soil surface wet for a longer period but consequently greater loss due to evaporation. Heavy application of water causes heavy deep percolation losses.

Selection of proper method of irrigation is important based on soil and crop characteristics. WUE is generally higher with sprinkler irrigation than surface method of irrigation. Drip irrigation also increases production and decrease the water use by a crop.

Among surface irrigation methods, WUE of crops increases in the order of wild flooding, border strip, check basin, basin and furrow irrigation.

7. Fertilizer application

WUE of crops invariably increases with the application of fertilizers on deficient soils under adequate soil moisture conditions. This is particularly with high yielding varieties and hybrids.

8. Weed control

Weeds due to their early establishment and a better root system are able to exhaust soil moisture more effectively than crop plants. Therefore, both yield and WUE are reduced. Controlling weeds is essential for higher WUE of crops.

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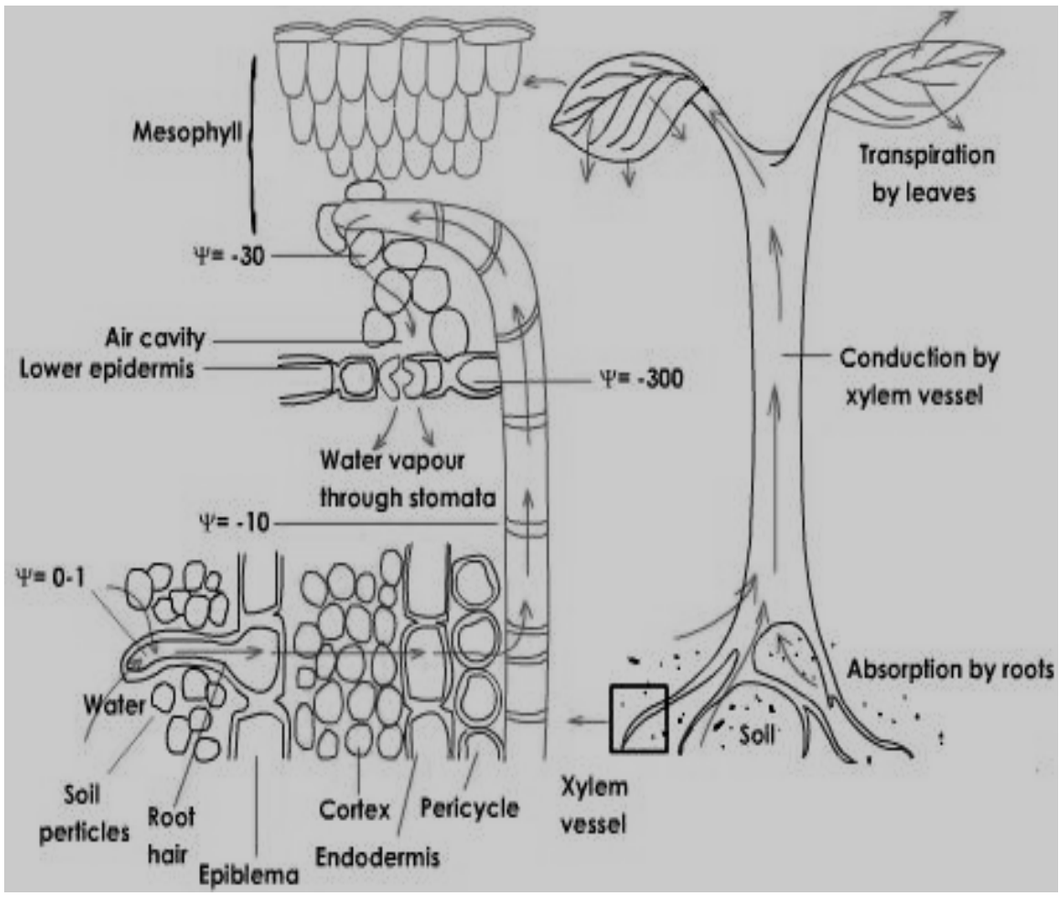
ANNEXURE



Movement of water towards more negative water potential

Lecture-9

PHOTOSYNTHESIS

9.1 DEFINITION: Photosynthesis is the process by which organisms convert light energy into chemical energy in the form of reducing power as NADPH and ATP. This reducing power is used to fix carbon dioxide as carbohydrates (sugars). In oxygenic photosynthetic organisms, including higher plants, the source of reducing equivalents is H_2O, releasing O_2 as a by product.

Thus, the overall reaction of oxygenic photosynthesis can be represented as.

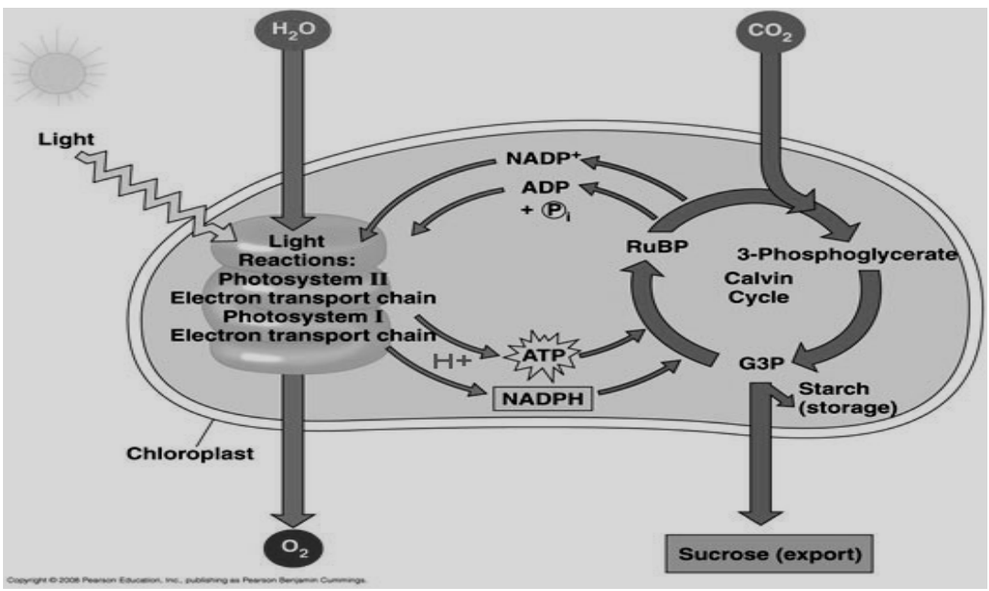
$$6CO_2 + 12\ H_2O \xrightarrow[\text{CHLOROPHYLL}]{\text{LIGHT}} C_6H_{12}O_6 + 6\ H_2O + 6\ O_2\uparrow$$

This equation is frequently represented by the simplified form:

$$CO_2 + 2\ H_2O \longrightarrow (CH_2O) + H_2O + O_2$$

Reactions of Photosynthesis:

During the normal functioning of the photosynthetic system, light serves to reduce nicotinamide-adenine dinucleotide phosphate (NADP). This in turn serves as a reducing agent for carbon fixation in the carbon reduction cycle. ATP is also formed during the electron flow from water to NADP, and it too used in carbon reduction. The chemical reactions in which water is oxidized to oxygen, NADP is reduced, and ATP is formed are generally known as the **light reactions** while the carbon reduction reactions are called the **dark reactions**.



All most all reactions up to NADP reduction takes place with in the thylakoids, while the carbon reduction rections take place in the aqueous region of chloroplast, the stroma. The following figure explains about the link between light reaction and dark reaction.

9.2 ENERGY SYNTHESIS:

Energy synthesis is the prime function of photosynthesis. It involves the role of various aspects like chloroplast and its structure, pigments present in chloroplast, Electro magnetic spectrum, photosynthetically active radiation, light interception by canopy, Capturing of light by pigment molecules, Light reaction (photolysis of water, electron transport between photo systems, generation of reducing power) and utilization of this reducing power in fixation of CO_2 to carbohydrates (dark reaction). Each of this aspect is briefly explained here.

9.2.1 CHLOROPLAST AND ITS STRUCTURE:

In photosynthetic eukaryotes, photosynthesis takes place in the sub cellular organelle known as chloroplast.(Fig.6)

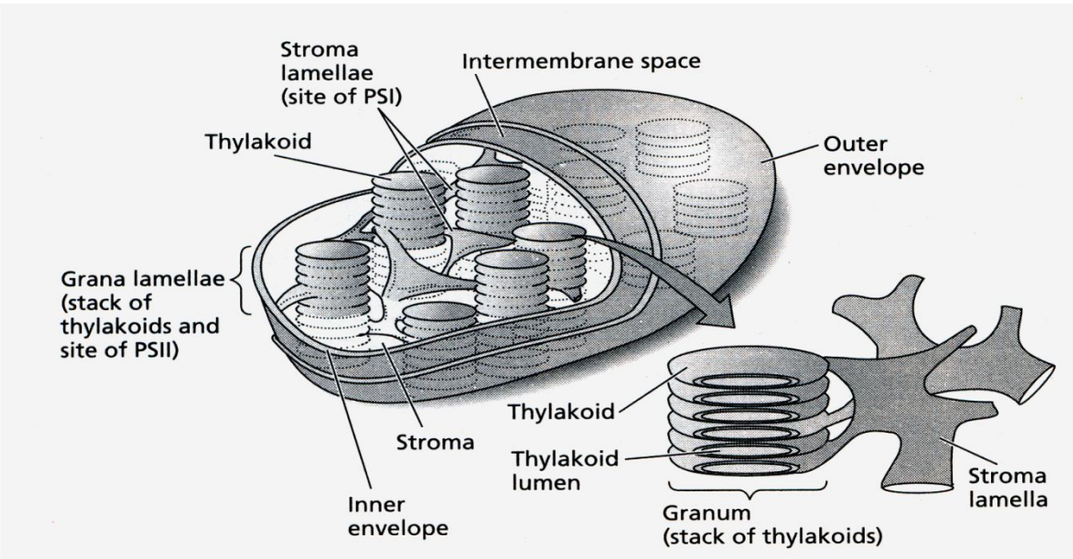


*Figure.6 is from reference 6

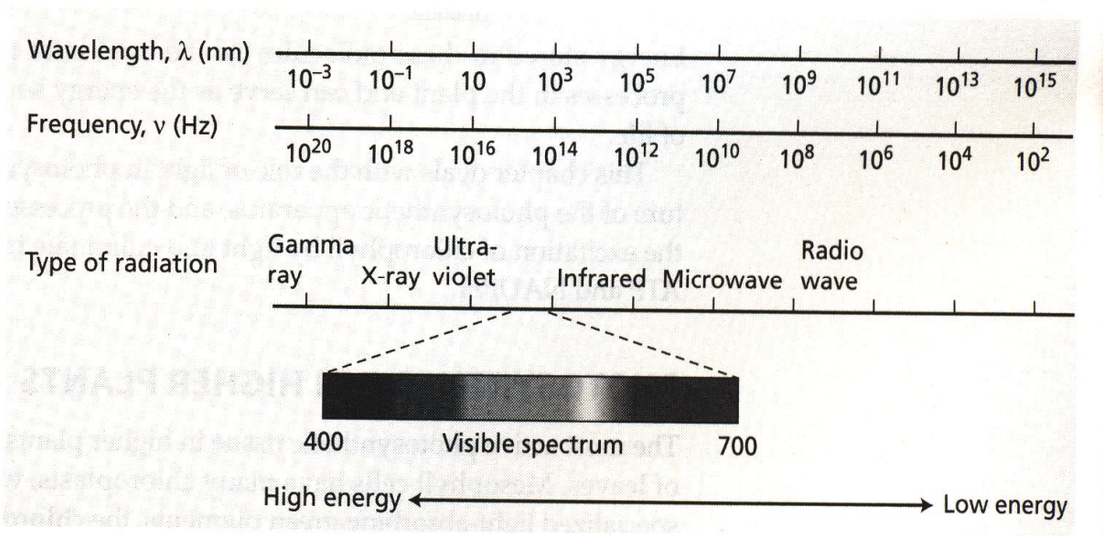
The most striking aspect of the structure of the chloroplast is this extensive system of the internal membranes known as **thylakoids**, (On these thylakoid bodies some round shaped photosynthetic units called **quantasomes** are presnt.) All the chlorophyll is contained with in this membrane system, which is the **site of the light reactions** of photosynthesis. The carbon reduction reactions or **dark reactions** which are catalyzed by water soluble enzymes, takes place in the **stroma**, the region of the chloroplast outside the thylakoids. Stroma forms the matrix of the chloroplast. In this portion of chloroplast, lamellae are loosely arranged. The lamellae which are found in this region are called stroma lamellae.

9.2.2 PHOTOSYNTHETIC PIGMENTS:

There are four different pigments in higher plants- two chlorophylls (Chl. A and Chl. B) and two carotenoids (carotene and xanthophyll). The chlorophylls are green in color whereas carotene and xanthophylls are orange and yellow, respectively. Magnesium present in chlorophyll molecule is crucial to the capture of light energy.

All organisms actually contain a mixture of more than one kind of pigment, each serving a specific function. As the energy of light absorbed by carotenoids is rapidly transferred to chlorophylls, carotenoids are termed as **accessory pigments**.

9.2.3 ELECTRO MAGNETIC SPECTRUM:

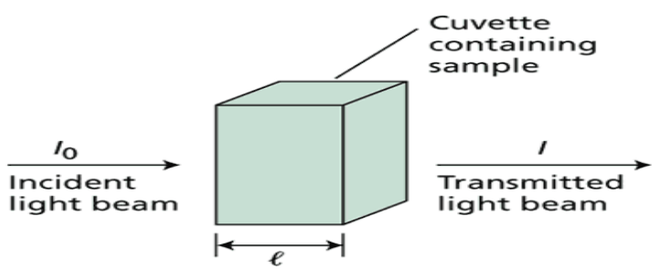


In the electromagnetic spectrum the wavelengths between 400-700nm is called as PHOTOSYNTHETICALLY ACTIVE RADIATION (PAR).

9.2.4 PRINCIPLE OF LIGHT ABSORPTION BY PLANTS:

The depression of light intensity within the plant community is mainly due to interception of light by leaves. The absorption of radiant energy by a plant community would follow the Lambert-Beer's Law.

Beer's Law states that the absorption of light by a solution is proportional to the concentration of the solution and the distance through which the light travels



Here the absorbance is defined as $A = \log I_0 / I$ where I_0 is the intensity of light that is incident on the sample and I is the intensity of light that is transmitted by the sample.

Lambert expressed this relationship mathematically as

$$I = I_0 e^{-KX}$$

Where I_0 = Incident light intensity

I = Intensity of the light after passing through the solution

K = Absorption coefficient.

X = Distance or path length .

Davidson and Philip modified this equation for absorption of energy by a plant canopy. They substituted LAI for x (i.e the path length). The hypothesized equation for absorption of radiant energy by a plant canopy then becomes

$$I = I_0 e^{-KA}$$

(or) $K = \log_e (I_0/I) / A$

The proportion of incident light that is intercepted by the canopy does not depend on leaf area index alone, but also on the architecture of the canopy. In a crop the leaves are not isolated, but are arranged in a canopy. The way they are arranged will affect the proportion of incident light that is intercepted by the crop canopy as a whole. Thus, the extinction coefficient (K) is a measure of the light intercepting efficiency of the leaf area. Leaf characters of importance in this respect are: leafEangle, leaf area, continuity of leaf layers etc.

9.2.5 THE TWO PHOTO SYSTEMS:

9.2.5.1 Evidence for the existence of PSI and PSII. (Red drop & emerson enhancement effect)

Experiments measuring the quantum yield (i.e. the number of O_2 molecules released for each Quanta absorbed) is approximately 0.1. The reciprocal of quantum yield is quantum requirement i.e. the number of quanta required for each O_2 molecule evolution. It is 10). If the quantum yield of photosynthesis is measured at different ranges of wave lengths most of the ranges are remarkably constant. However at the extreme red edge of the chlorophyll absorption (>680nm), the yield drops drastically. The phenomenon is known as **RED DROP**.

Conclusion: It is proposed that at this wave length only one photosystem remains in operation. As another photosystem is not able function at this wavelength quantum yield drops.

Another puzzling experimental result was the enhancement effect, discovered by Emerson. The rate of photosynthesis was measured separately with light of two different wave lengths and then the two beams were used simultaneously. When exposed to a wavelength more than 680 nm (far red region) a specific rate of photosynthesis was observed. Likewise when the exposure was given at wavelengths less than 680 nm some other effect was observed. When the system was exposed to the light of both wavelengths simultaneously, the effect on photosynthesis exceeded the sum of the two effects caused separately. This provided evidence that the two pigment systems worked in co-operation with each other and the increase in photosynthesis was due to synergism. This phenomenon is known as **Emerson Enhancement effect**. This experiment also explained that, there exist two photo systems (PS-I & PS-II)

Based on these two observations Hill and Fay Bendall (1960) proposed that light reactions of photosynthesis involve two photochemical events.

9.2.5.2 PHOTOSYSTEM I & II:

The reaction centre chlorophyll of **photo system I** absorbs maximally at 700 nm in its reduced state. Accordingly, it is named **P 700** (the P stands for Pigments), the reaction centre chlorophyll of **photo system II** absorbs maximum at 680 nm (**P 680**)so its reaction centre chlorophylls and associated electron transport proteins is located predominantly in the stacked regions of the grana lamellae. Whereas the photo system I, its associated antenna pigments, ATP synthase enzymes are found exclusively in the stroma lamella and at the edges of the grana lamellae. (At a wavelength grater than 680 nm PS-II can not operate. Therefore, quantum yield decreased beyond 680 nm. This is what red drop is.)

Photo system I contains **large amount of chlorophyll a**, a small amount of chlorophyll b and some B carotene. Photo system II also contains chlorophyll a, B carotene but a **large amount of chlorophyll b**.

9.2.6 LIGHT REACTIONS:

Almost all the chemical processes that make up the light reactions of photosynthesis are carried out by four protein complexes; photo system II (P 680), the cytochrome b-f complexes, photo system I (P 700) and the ATP synthase.

SEQUENCE OF LIGHT REACTIONS:

9.2.6.1 Water is exidized to oxygen by Photo system II:

The chemical reaction by which water is oxidized is given by the following equation.

$$2H_2O \longrightarrow O_2 + 4H^+ + 4e^-$$

This equation indicates that four electrons are removed from two water molecules, generating an oxygen molecule and four hydrogen ions.

Robert Hill demonstrated that isolated chloroplasts evolved Oxygen when they were illuminated in the presence of suitable electron acceptor, such as ferricyanide. The ferricyanide is reduced to ferrocyanide by photolysis of water .This reaction is now called as **HILL REACTION** and it explains that water is used as a source of electrons for CO_2 fixation and Oxygen is evolved as a by product.

The protons produced by oxidation of water is released into the lumen of the thylakoid, not directly into the stromal compartment. These protons are eventually released from the lumen to the stroma through the process of ATP synthesis. In this way the electro chemical potential formed by the release of protons during oxidation of water contributes to ATP formation.

Water Oxidation System: Manganese (Mn) is an essential cofactor in the water oxidizing process. Analytical experiments indicate that four Mn ions are associated with oxygen evolving complex. Other experiments have shown that cl and Ca ions are also essential for O_2 evolution.

9.2.6.2 ELECTRON TRANSPORT AND PHOSPHORYLATION:

The Z scheme (named because of its shape) illustrates electron transport and the production of NADPH and ATP in chloroplasts.

9.2.6.2.1 <u>NON CYCLIC PHOTOPHOSPORYLATION:</u>

Non cyclic photophosphorylation involves integration of two photo systems (PS-I and PS-II). This is one of the means of ATP production in chloroplasts.

Non-Cyclic	:	Electrons follows a non cyclic track.
Photo	:	it is the light energy that drives electrons along this Track.
Phosphorylation	:	As electrons are driven along the track ADP is phosphorylated to yield ATP.

This can also be termed as non-cyclic electron transport to refer to the manner of electron flow during the process.

MECHANISM:

The primary flow of electrons within a given granum thylakoid may be initiated almost simultaneously for each photo system (PS I and PS II).

After excitation of P_{700} the electrons are passed on to a chlorophyll molecule (Ao). The electrons are then passed to series of Iron-sulfur proteins (Fe-S) and finally to ferridoxin (Fd). The ferridoxin-NADP reductase serves to reduce NADP to NADPH which is used in the Calvin cycle to reduce Co_2.(Fig. 7)

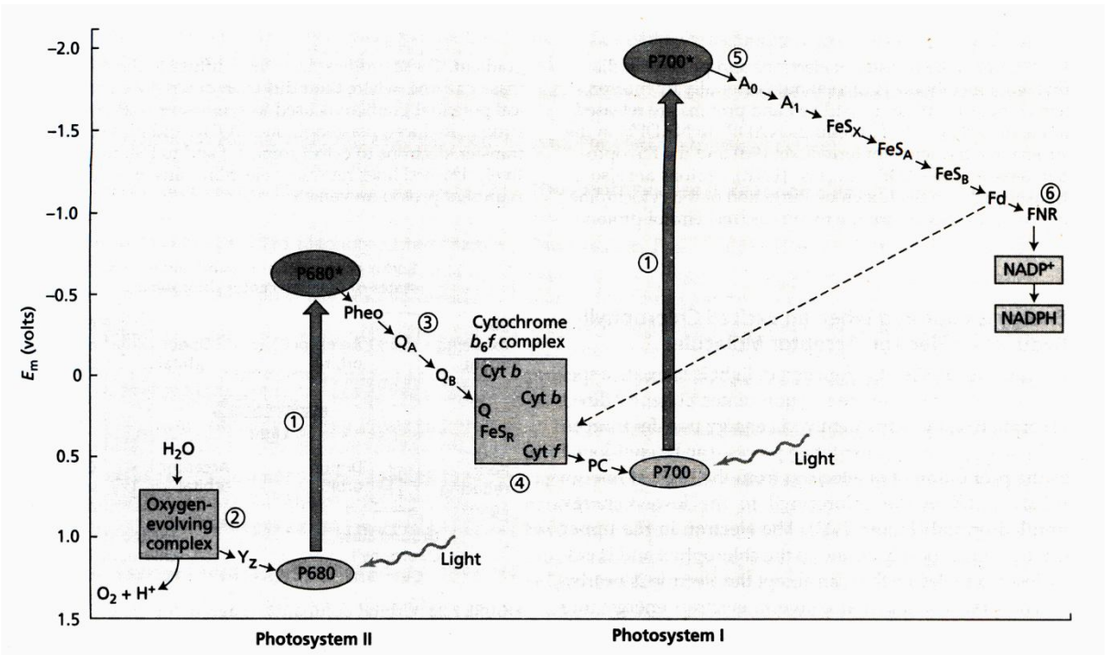
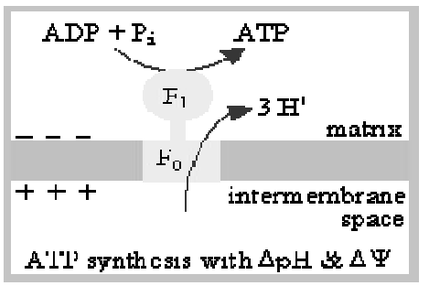


FIGURE Detailed Z scheme for O_2-evolving photosynthetic organisms. The redox carriers are placed at their midpoint redox potentials (at pH 7). (1) The vertical arrows represent photon absorption by the reaction center chlorophylls: P680 for photosystem II (PSII) and P700 for photosystem I (PSI). The excited PSII reaction center chlorophyll, P680*, transfers an electron to pheophytin (Pheo). (2) On the oxidizing side of PSII (to the left of the arrow joining P680 with P680*), P680 oxidized by light is re-reduced by Y_z, that has received electrons from oxidation of water. (3) On the reducing side of PSII (to the right of the arrow joining P680 with P680*), pheophytin transfers electrons to the acceptors Q_A and Q_B, which are plastoquinones. (4) The cytochrome b_6f complex transfers electrons to plastocyanin (PC), a soluble protein, which in turn reduces $P700^+$ (oxidized P700). (5) The acceptor of electrons from P700* (A_0) is thought to be a chlorophyll, and the next acceptor (A_1) is a quinone. A series of membrane-bound iron–sulfur proteins (FeS_X, FeS_A, and FeS_B) transfers electrons to soluble ferredoxin (Fd). (6) The soluble flavoprotein ferredoxin–NADP reductase (FNR) reduces $NADP^+$ to NADPH, which is used in the Calvin cycle to reduce CO_2 (see Chapter 8). The dashed line indicates cyclic electron flow around PSI. (After Blankenship and Prince 1985.)

*Figure.7 is from reference 6

The transfer of electrons to NADP creates debit (commonly referred to as a hole) in photo system I. However this deficit is made up by the excitation of P_{680} of photo system II. The excited P680 of PS II transfers electrons to pheophytin, plastoquinones, and cyt b_6-f complex. Cytochrome b_6-f complex transfers electrons to plastocyanin (PC), which in turn reduces P_{700}* (excited P700). The hole created in photo system II is filled by-electrons that are derived from the oxidation of water.

In addition to the energy stored as redox equivalents (NADPH) by the light reactions a portion of the photons energy is utilized for the synthesis of ATP during the transfer of electrons between plaotoquinone and cyt b_6-f complex. This phenomenon of synthesis of ATP in light reactions of photosynthesis is known as **photophosphorylation**. It is now widely accepted that photophosphorylation works via the **chemi-osmotic mechanism** first proposed in 1960 by **Peter Mitchell**.



The basic principle of chemi-osmosis is that in concentration differences and electrical potential differences across the membranes are a source of free energy that can be utilized by the cell for the synthesis of ATP. In the light reactions electron flow is coupled to proton translocation, creating transmembrane proton motive force (pmf). The energy in the proton motive force is then used for synthesis of ATP by the enzyme called ATP synthase.

This synthesis of ATP coupled to a linear flow of electrons from water to NADP is called as non cyclic photophosphorylation. The precise number of ATP molecules formed in non-cyclic photophorylation is unclear although it is generally thought **two** per O_2 evolved.

9.2.6.2.2 <u>CYCLIC PHOTOPHOSPHORYLATION:</u>

The cyclic photophosphorylation operates when chloroplasts are illuminated with wave lengths of light greater than 680nm. Under these circumstances only photo system I is activated and electrons are not removed from H_2O. When the flow of electrons from H_2O is stopped, non cyclic assimilation retarded, oxidized NADP is no longer available as an electron acceptor. Activation of photo system I by wave lengths of light greater than 680 nm causes electron to flow from P_{700} to chlorophyll molecule and Ferridoxin. Then the electrons instead of pass on to NADP return back to P_{700} via cyt b_6-f complex, plastoquinone and plastocyanin.

Cyclic transport system is likely to result in the synthesis of ATP at two locations. One is between Fe-s protein and cyt-b_6 complex and another between cyt-b_6 and cytochrome f.

Significance

Evidence for the operation of cyclic electron transport in C_3 plants *in vivo* is limited but it has been demonstrated under physiological conditions *in vivo* in C_4 plants where there is an additional ATP requirement in their carbon fixation pathway. It may also play an important role in the synthesis of ATP required for protein synthesis during PS II repair following photo inhibition.

9.2.6.2.3 PSEUDO CYCLIC PHOSPHORYLATION:

Another source of generation of ATP is that electrons might be transferred from ferridoxin back to oxygen reducing it to water. It is possible that this process might also involve an electron transport chain and produce ATP. Here the electron that is cycled back to reduce molecular oxygen to water is not the same that is released from the water. Hence it is called as pseudo cyclic phosphorylation.

9.2.7 CARBON DIOXIDE FIXATION /DARK REACTIONS OF PHOTOSYNTHESIS:

During the light reactions of photosynthesis, the photochemical oxidation of water to molecular oxygen is coupled to the generation of reduced pyridine nucleotide (NADPH) and ATP. The reactions associated with the reduction of CO_2 to carbohydrate are coupled to the consumption of NADPH and ATP. These reactions are referred to as the Dark reactions of the photosynthesis.

9.2.7.1 The C3 Cycle (C3 Photosynthetic Carbon Reduction Cycle)

The PCR cycle is sometimes referred to as the Calvin cycle in honor of its discoverer, the American biochemist Melvin Calvin, other pathways associated with the photosynthetic fixation of CO_2, such as the C_4 photosynthetic carbon assimilation (PCA) cycle and the C2 photo respiratory carbon oxidation cycle (PCO), are either auxiliary to or dependent on the basis PCR cycle.

In the C_3, PCR cycle, carbon dioxide from the atmosphere and water are enzymatically combined with a five-carbon acceptor molecule to generate two molecules of a three carbon intermediate. These intermediates are reduced to carbohydrate using the photo chemically generated ATP and NADPH in the light reactions. The cycle is completed by the generation of five-carbon acceptor.(Fig.8)

The C3 PCR cycle proceeds in three stages:

1. **Carboxylation** of the CO_2 acceptor, ribulose 1,5 – bisphosphate, to form 2 molecules of 3. Phosphoglycerate, the first stable intermediate of the PCR cycle.
2. **Reduction** of this carboxylic acid to a carbohydrate in the form glyceraldehydes 3- phosphate.
3. **Regeneration** of CO_2 acceptor, ribulose 1.5-biophosphate from glyceraldehydes 3-phosphate.

The Carboxylation of Ribulose Bisphosphate:

CO_2 enters the PCR cycle by reacting with ribulose 1,5-bisphosphate to yield two molecules of 3-phosphoglycerate, a reaction that is catalyzed by the chloroplast enzyme ribulose bisphosphate carboxylase/oxygenase, referred to by the acronym **RUBISCO**.

The reduction step of the C_3 PCR cycle:

In this stage, the 3-phosphoglycerate formed as a result of the carboxylation of RuBP (Ribulose 1,5-bisphosphate) is first phosphorylated to 1,3-bis phospho glycerate by the ATP generated in the light reactions and is then reduced to glyceraldehyde 3-phosphate, using the NADPH generated by the light reactions. The chloroplast enzyme NADP-glyceraldehyde 3-phosphate dehydrogenase catalyzes this step.

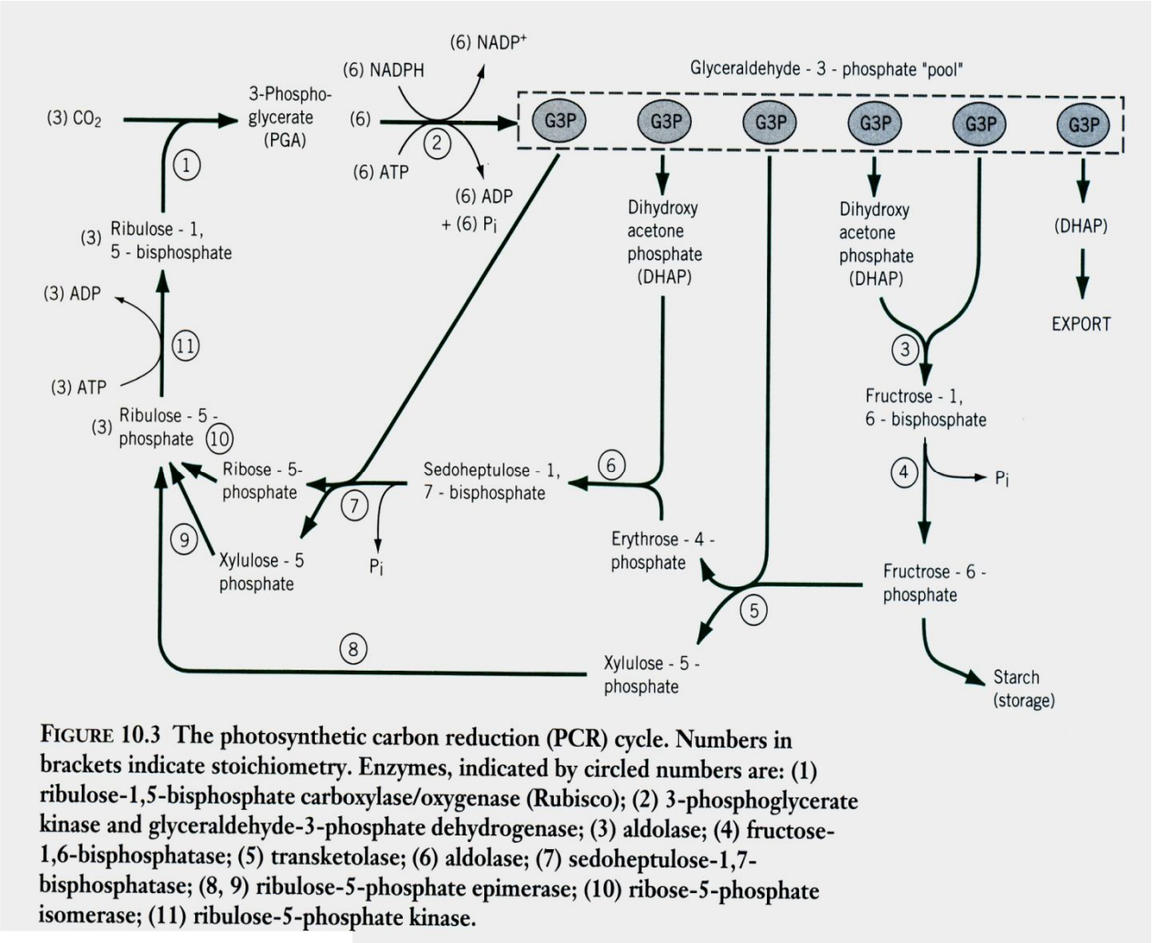


FIGURE 10.3 The photosynthetic carbon reduction (PCR) cycle. Numbers in brackets indicate stoichiometry. Enzymes, indicated by circled numbers are: (1) ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco); (2) 3-phosphoglycerate kinase and glyceraldehyde-3-phosphate dehydrogenase; (3) aldolase; (4) fructose-1,6-bisphosphatase; (5) transketolase; (6) aldolase; (7) sedoheptulose-1,7-bisphosphatase; (8, 9) ribulose-5-phosphate epimerase; (10) ribose-5-phosphate isomerase; (11) ribulose-5-phosphate kinase.

*Figure.8 is from reference 5

Regeneration of Ribulose 1, 5 -Bisphosphate:

One molecule of glyceraldehyde 3-phosphate is converted to dihydroxy acetone 3-phosphate (DHAP). The DHAP then undergoes aldol condensation with a molecule of glyceraldehydes 3-phosphate to form fructose 1,6-bis phosphate. This product is hydrolyzed to fructose 6 phosphate. This fructose 6 phosphate combines with third molecule of glyceraldehyde 3-phosphate to give erythrose 4-phosphate and xylulose 5-phosphate. This reaction is catalyzed by transketolase.

Erythrose 4-phosphate then combines with DHAP to yield a seven carbon sugar, sedoheptulose 1,7-bisphosphate which is further hydrolyzed to give sedoheptulose 7-phosphate. Sedoheptulose 7-phosphate donates a two carbon unit to the fifth molecule of glyceraldehyde 3-phosphate and produce ribose 5-phosphate

and xylulose 5-phosphate as its products. Two molecules of xylulose 5-phosphate are epimerized to give ribulose 5-phosphate. The third molecule of ribulose 5-phosphate is formed by the isomerization of ribose 5-phosphate. Finally, ribulose 5-phosphate is phosphorylated with ATP. Thus generating CO_2 acceptor ribulose 1,5-bisphosphate.

Energy requirement: In order to synthesize the equivalent of 1 molecule of hexose sugar, 6 molecules of CO_2 are fixed at the expense of 18 ATP and 12 NADPH. In other words, the PCR cycle consumes 2 molecules of NADPH and 3 molecules of ATP for every CO_2 fixed.

$6CO_2+11H_2O+12$ NADPH + 18ATF $\longrightarrow$ Fructose- 6- P +12 NADP+6 H^+ + 18 ADP + 17 Pi

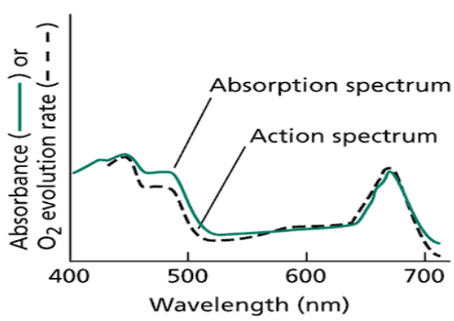
****

ANNEXURE

Apart from stroma lamellae ribosomes serving as sites for the protein synthesis are also found scattered in this region. Lipid, protein and nucleic acid metabolisms are also found in this region.

Chlorophyll molecule is a typical porphyrin derivative possessing a cyclic tetrapyrollic structure in which one pyrrole ring is partially reduced. The tetrapyrorolic nucleus contains a **non-ionic magnesium** atom held by two covalent and two coordinate bonds in the centre of the molecule. Magnesium is crucial to the capture of light energy. The presence of methyl group (CH_3) at position 3 in the second pyrrole ring makes chlorophyll-a and when it is replaced by formyl group (-CHO) it forms chlorophyll-b.

		Chemical formula	Absorption peaks
1	Cholorophyll-a	$C_{55}H_{72}O_5N_4Mg$	662, 430nm
2	Cholorophyll-b	$C_{55}H_{70}O_6N_4Mg$	642, 453nm



An action spectrum is measured by plotting a response to light such as oxygen evolution, as a function of wavelength. If the pigments used to obtain the absorption spectrum are the same as those that cause the response, the absorption and action spectra will match. In the example shown here, the action spectrum for oxygen evolution matches the absorption spectrum of intact chloroplasts quite well, indicating that light absorption by the chlorophylls mediates oxygen evolution.

***For further information see reference.6**

Lecture-10

PHOTOSYNTHESIS

10.1 THE C_4 PHOTOSYNTHETIC CARBON ASSIMILATION (PCA) CYCLE

Hal Hatch and Roger Slack elucidated what is now known as the C_4 PCA cycle. Using sugarcane leaves, they established that the C_4 acids malic acid and aspartic acid were the first stable detectable intermediates of photosynthesis in leaves of C_4 plants. The primary carboxylation in these leaves was not catalyzed by Rubisco but by PEP carboxylase.

The basic C_4 PCA cycle consists of four stages.

1. Assimilation of CO_2 involving carboxylation of phosphoenol pyruvate (PEP) in the mesophyll cells to form C_4 acids (Malate and / or aspartate)
2. Transport of C4 acids to the bundle sheath cells.
3. Decarboxylation of C_4 acids within in the bundle sheath cells and generation of CO_2 which is reduced to carbohydrate via the C_3 PCR cycle.
4. Transport of the C_3 acid formed by the decarboxylation (pyruvate or alanine) back to the mesophyll cell and regeneration of the CO_2 acceptor, phosphoenol pyruvate.

Anatomical differences

A cross section of a typical C_3 leaf reveals essentially one type of photosynthetic, chloroplast containing cell, the mesophyll. In contrast, a typical C_4 leaf has two distinct chloroplast containing cell types, the mesophyll and the bundle sheath cells. The extensive network of **Plasmodesmata** connects mesophyll and bundle sheath cells, providing a pathway for the flow of metabolites between cells. This is called as **KRANZ ANATOMY**. (Fig.9)

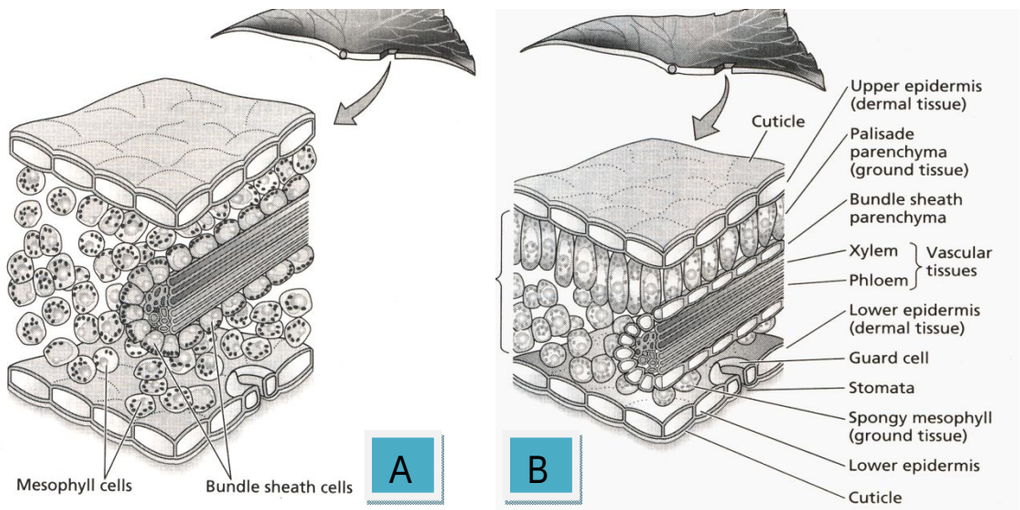


*Figure 9 is from reference 6

The figure ‘A’ shows that in a C_4 leaf both mesophyll and bundle sheath cells possess chloroplasts.

Discovered in tropical grasses (e.g., sugarcane and maize), the C4 cycle is now known to occur in 16 families of both monocortyledons and dicotyledons, and it is particularly prominent in Gramineae (sugarcane, com, sorghum), Chenopodiaceae (Atriplex), and Cyperaceae (sedges). About 1% of the characterized species have C4 metabolism.

The primary carboxylatiion reaction, catalyzed by PEP carboxylase, which is common to all three variants, occurs in the cytosol of the mesoophyll chloroplasts by NADPH using NADP malate dehydrogenase. The malate formed enters the chloroplast of the bundle sheath cell and there undergoes oxidative decarboxylation, yielding pyruvate. The CO_2 released within the bundle sheath cells is converted to carbohydrate by the Calvin cycle. (Fig.10)

The residual C3 acid is transported back to the mesophyll as pyruvate and converted to phosphoenolpyruvate in the mesophyll chloroplast.

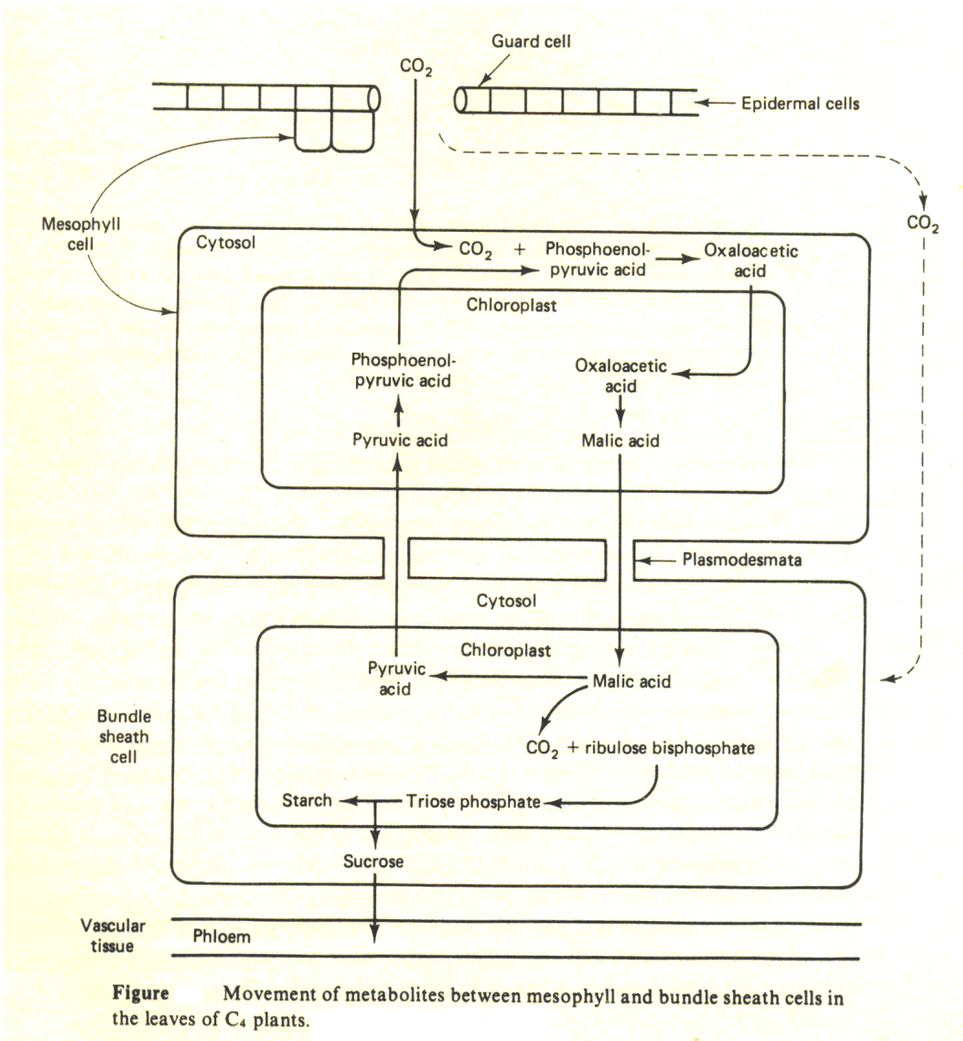


Figure Movement of metabolites between mesophyll and bundle sheath cells in the leaves of C_4 plants.

*Figure 10 is from reference 7

10.1.1 There are three variants in PCR cycle of C_4 plants:

Variant Name	Principal C4 acid transported to the bundle sheath cells	Decarboxylating enzyme	Examples.
NADP – ME	Malate	NADP-dependent Malic enzyme	Maize, Sugarcane, Sorghum
NAD-ME	Aspartate	NAD-dependent Malic enzyme	Millet, Pigweed, Panicum milliaceum (variga) Amaranthus
PEP-CK	Aspartate	Phosphoenol pyruvate carboxy kinase	Guinea grass (Panicum maximum), chloris gayana

These variants differ principally in the C_4 acid transported into the bundle sheath cells and decarboxylating enzyme.(Fig.11)

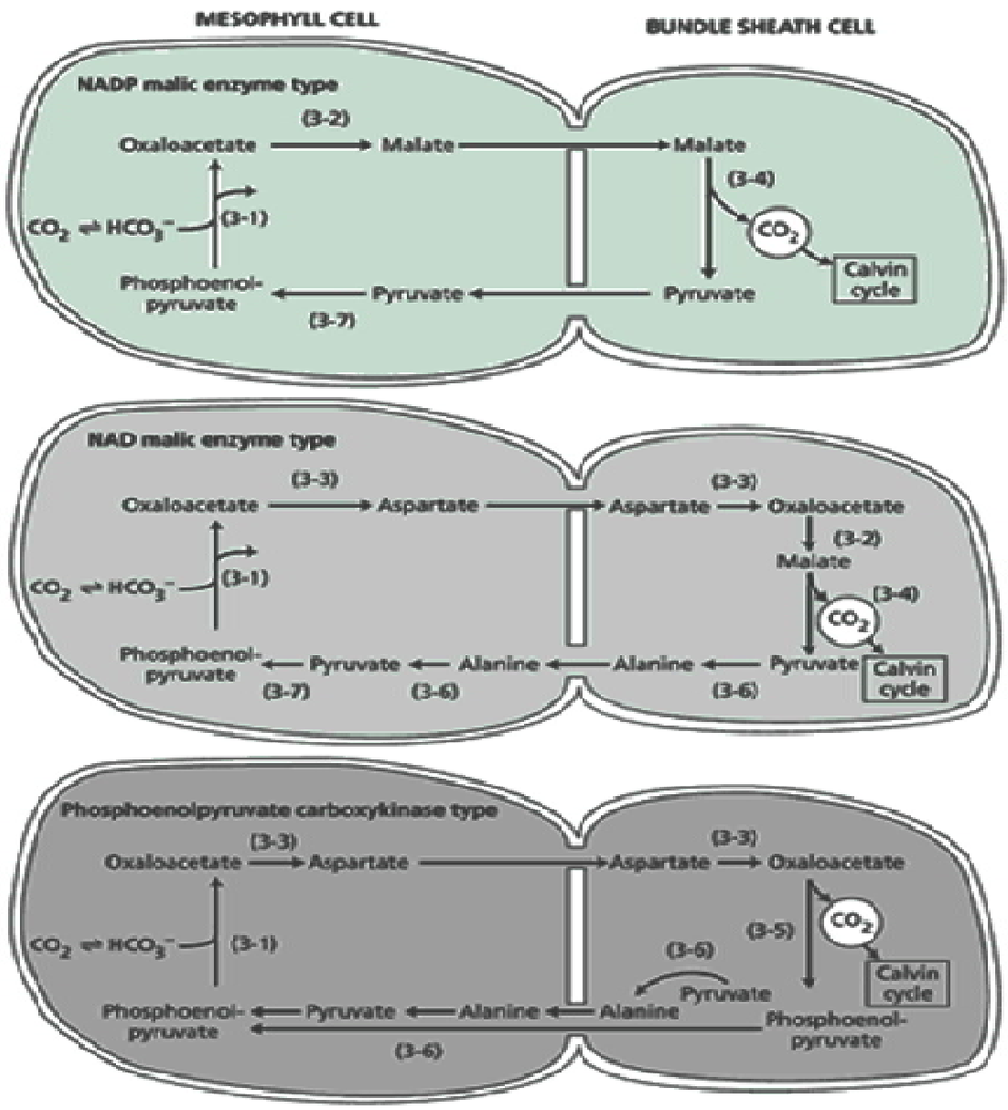


*Figure 11 is from reference 6

A) NADP-ME Type

The primary carboxylation reaction occurs in the cytosol of mesophyll cells and is catalyzed by phosphoenol pyruvate carboxylase (PEP carboxylase) using HCO^{3-} rather than CO_2 as a substrate.

Oxalo acetate formed in this reaction rapidly reduced to malate in the mesophyll chloroplasts by NADPH. The C_4 acid (Malate) then transported to the chloroplasts of bundle sheath cells and undergoes oxidative decarboxylation by NADP-ME. The CO_2 released within the bundle sheath cells is reduced to carbohydrate by the PCR cycle. The C_3 acid (Pyruvate) formed in the decarboxylation is transported back to the mesophyll. In the final step of PCA cycle, the pyruvate is converted to phosphoenol pyruvate with in the mesophyll chloroplast.

B) NAD-ME Type :The primary carboxylation reaction occurs in the cytosol of the mesophyll cells and is catalyzed by PEP carboxylase using HCO^{3-}.

Oxalo acetate formed undergoes transamination in the cytosol, with glutamate as the amino donor and forms into Aspartate. The C_4 acid (Aspartate) then transported to the bundle sheath cells. In the bundle sheath cells, the asparatate is first reconverted into oxalo acetate by transamination in the mitochondria. The oxalo acetate in the mitochondria is then reduced and decarboxylated by the NAD-Malic enzyme. The CO_2 released within the bundles sheath cells is reduced to carbohydrate by PCR cycle. The C3 acid pyruvate undergoes transamination and formed in to alanine which will be transported back to mesophyll cells.In the measophyll cytosol alanine appears to be converted to pyruvate via transamination. In the final step the pyruvate is converted to phosphoenol pyruvate within the mesophyll chloroplast.

C)PEP-CK type

The primary carboxylation reaction occurs in cytosol of mesophyll cells and is catalyzed by PEP carboxylase using HCO_3^-. The Oxalo acetate undergoes transamination and formed into aspartate a C_4 acid. The C_4 acid then transported to the bundle sheath cell and is reconverted into oxalo acetate. The oxalo acetate is decarboxylated by PEP carboxy knase and forms into phosphoenol pyruvate. The PEP then transported to mesophyll cells.

10.1.2 Significance of C_4 Photosynthesis

C_4 PCA cycle effectively shuttles CO_2 from the atmosphere into the bundle sheath cells. This transport process generates a much higher concentration of CO_2 in the bundle sheath cells than would occur in equilibrium with the external atmosphere. This elevated concentration of CO_2 at the site of carboxylation of ribulose 1,5 biphosphate by RUBISCO results in the suppression of ribulose biphosphate oxygenation or Photorespiration. This is called as CO_2 concentration mechanism.

10.1.3 Energy Requirement

One interesting feature of the cycle is that the enzyme that catalyzes the regeneration of phosho enol pyruvate by the enzyme pyruvate-orthophosphate dikinase, consumes two “high-energy” phosphate bonds through the conversion of ATP to AMP. There by, explaining the high energy requirement of C_4 cycle.

Total energy requirement for fixing one molecule of CO_2 in C_4 plants is 5 ATP plus 2 NADPH. This includes the cost of concentrating CO_2 with in the bundle sheath cell i.e. 2ATP per CO_2

10.2 CRASSULACEAN ACID METABOLISM (CAM)

CAM is an acronym for crassulacean acid metabolism, but the mechanism is not restricted to the family crassulaceae alone, like the C_4 PCA cycle, it is found in many angiosperm families.

The CAM mechanism is similar in many respects to the C_4 PCA cycle, but differs from it in two important features.

1) In C_4 plants the formation of C_4 acids is spatially, but not temporally, separated from the decarboxylation of the C_4 acids and re fixation of the resulting CO_2 by the PCR cycle. In CAM plants, the formation of C_4 acids is temporally but not spatially, separated from decarboxylation and refixation. CAM plants lack the specialized leaf anatomy **kranz leaf anatomy** typical of C_4 plants.

2) CAM plants open their stomata during the cool, desert nights and closed during the hot, dry days. This minimizes water loss. The CO_2 is assimilated at night. (Fig.12)

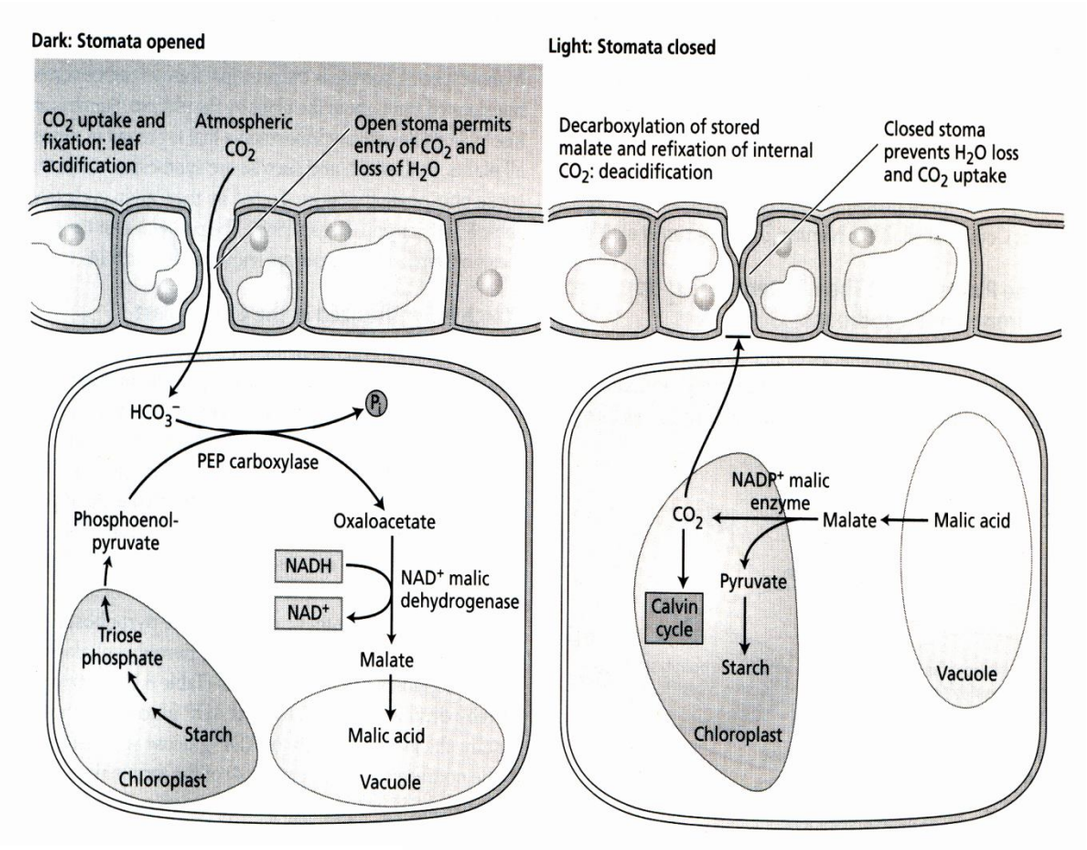


*Figure 12 is from reference 6

The CO_2 assimilation is accomplished by carboxylation of phosphoenol pyruvate to oxalo acetate, which is then reduced to malate. The PEP originates from the breakdown of starch and other sugars by the glycolytic pathway. The C_4 acid accumulates as malic acid in large vacuoles. The accumulation of substantial amounts of malic acid has long been recognized as **dark acidification of leaf**.

With the onset of day, the stomata close, preventing loss of water and further acquisition of CO_2. The leaf cells become deacidified as the reserves of vacuolar malic acid are consumed. Decarboxylation is achieved by the action of NADP malic enzyme on malate. Because the stomata are closed, the internally released CO_2 cannot escape from the leaf and instead is reduced to carbohydrate by operation of the C_3 PCR cycle. The C_3 acid remaining after decarboxylation is thought to be converted into starch or sucrose thus recovering the original starting material.

10.2.1 Significance:

The CAM mechanism enables plants to maximize their water – use efficiency due to **skoto active opening of stomata.** Typically a CAM plant loses 50-100 g of water for every gram of CO_2 gained, compared with the values of 250-300 and 400-500g for C_4 and C_3 plants respectively. Thus CAM plants are specially adapted to arid environment. Like in C_4 plants, the elevated internal concentration of CO_2 effectively suppresses the photo respiratory oxygenation of ribulose biphosphate and favors carboxylation.

10.3 MEASUREMENT OF PHOTOSYNTHESIS:

There are different methods by which photosynthesis can be measured.

A. Assay of Chloroplast activity (Hill reaction)

Either intact or isolated chloroplasts of leaf fragments evolve oxygen and electrons during photosynthesis. Photosynthesis hence is measured by the changes in the absorption spectra of 2,6-di chloro phenol indo phenol (DCPIP) which is reduced by the evolved electrons. It represents chloroplast activity. This method is convenient to identify photosynthetic inhibitors.

B. Gas exchange Measurement (Infra – red Gas Analysis IRGA)

Photosynthetic gas exchange refers to the fluxes or flows of CO_2 between t**he plant** and environment.

Infrared gas analysis is the most popular, accurate and simple technique determining photosynthetic or respiratory CO_2 exchange technique in air. It is based on the principle that the hetero atomic gas molecules typically absorb radiation at specific infrared wave bands and that each gas has got a characteristic absorption spectrum and not interfered by gas molecules of identical atoms. Hence IRGA can be used for accurate determination of concentrations of CO_2 uptake by small leaves.

C. Monitoring with $^{14}CO_2$: Photosynthesis true to the field, is measured by

a) Exposing Photosynthetic tissue to $^{14}CO_2$

In this photosynthetic tissue is exposed to a gas of $^{14}CO_2$. By killing the tissue after a definite time $^{14}CO_2$ fixed is determined and the rate of Photosynthesis is estimated.

b) Exposing leaf to a gas mixture in a closed space:

In this method the leaf is exposed in a closed space to a gas mixture containing the carbon isotopes and the rate of decrease of radioactivity of the gas is measured by a built in β- counter.

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Lecture-11

PHOTOSYNTHESIS

11.1 THE C_2 PHOTO RESPIRATORY CARBON OXIDATION (PCO) CYCLE

All land plants have an additional metabolic activity and loose a considerable amounts of their photosynthates as CO_2 with in a few seconds of its being fixed.It occurs when plants are illuminated. This process of CO_2 release is light dependent and because of its otherwise superficial resemblance to respiration, it has been given the name Photorespiration. During the process O_2 is consumed and CO_2 is generated in light. It is independent of mitochondrial respiration but occurs in chloroplasts and peroxisomes and only a few reactions occur in mitochondria. However, unlike true respiration it performs no useful function in making energy available to the plant. Instead its occurrence leads to loss of energy and this is responsible for a considerable reduction in the yield of many of the crops.

Definition: Photorespiration means evolution of CO_2 by green leaves in light.

11.1.1 Mechanism:

RuBISCO is a by **bifunctional enzyme** capable of carboxylation as well as oxygenation of RuBP. The operation of the PCO cycle involves cooperative interactions between three organelles, Chloroplasts, mitochiondria and peroxisomes.(Fig.13)

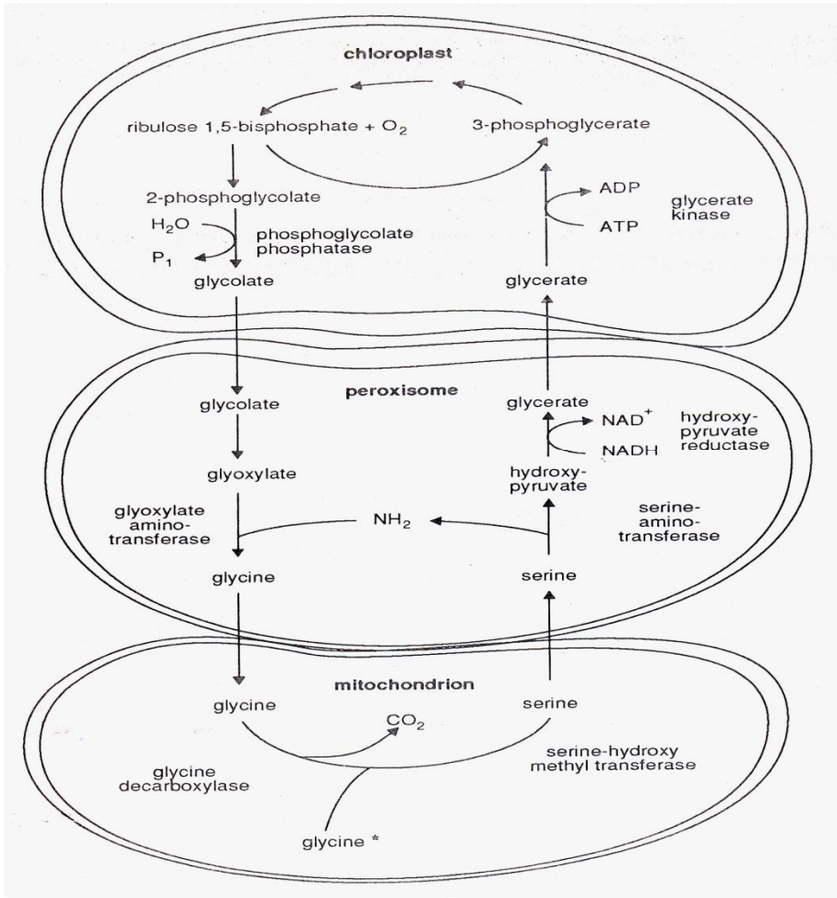


Figure.13

The oxygenation of RuBP results in the formation of 2-phosphoglycolate and 3-phosphoglycerate.The phospho glycolate is rapidly hydrolyzed to glycolate by a specific enzyme phosphatase. Glycolate leaves the chloroplast and diffuses to peroxisome. There it is oxidized by glycolate oxidase to glyoxylate and hydrogen peroxide. The peroxide is destroyed by the action of catalase and glyoxylate undergoes transamination and the product is the amino acid **glycine**.

Glycine leaves the peroxisomes and enters mitochondria, where two molecules of glycine are converted to serine. **Glycine is thus, the immediate source of the photo respiratory CO_2.** Serine leaves the mitochondrion and enters peroxisome, where it is converted first by transamination to hydroxy pyruvate and then by reduction to glycerate.

Finally, glycerate reenters the chloroplast, where it is phosphorylated to yield 3-phosphoglycerate.

11.1.2 Significance:

Photorespiration is a response to the low CO_2/O_2 ratios prevalent in the present day atmosphere and has no functional role. Another possible explanation is that photorespiration is necessary under conditions of high light intensities and low CO_2 concentration (e.g. when stomata are closed because of water stress) to dissipate excess ATP and reducing power from the light reactions, thus preventing damage to the photosynthetic apparatus. Today, there is no conclusive evidence for the role of photorespiration in the carbon metabolism of the leaf.

Many plants do not photo respire, this is not because that RUBISCO have different properties, rather, it is a consequence of interesting mechanisms that concentrate CO_2 in the RUBISCO environment is in the case of C_4 and CAM plants. (CO_2 concentration mechanism)

11.1.3 Energy requirement

Oxygenation of ribulose bis phosphate and operation of PCO cycle consumes 2ATP and 2.5 NADH for each ribulose bisphosphate oxygenated.

11.2 FACTORS AFFECTING PHOTOSYNTHESIS

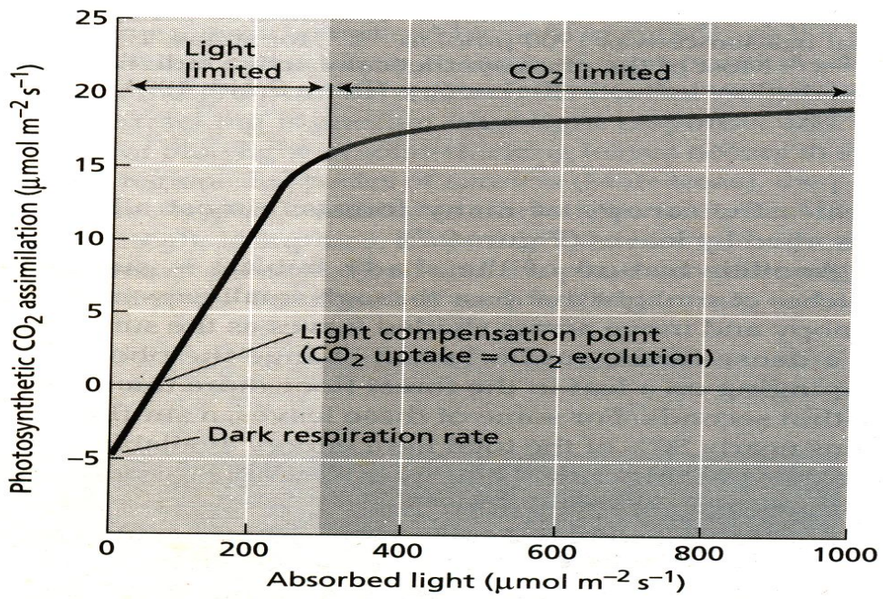
Many plant and environmental factors influence photosynthesis such as light, CO_2, temperature, availability of water as well as plant age and genetics.

11.2.1 LIGHT

The liner portion of light response curves of photosynthesis represent the part of the process in which photosynthesis is strictly light limited. Under this condition, each increment in light elicits a proportional increase in photosynthetic rate, resulting in a

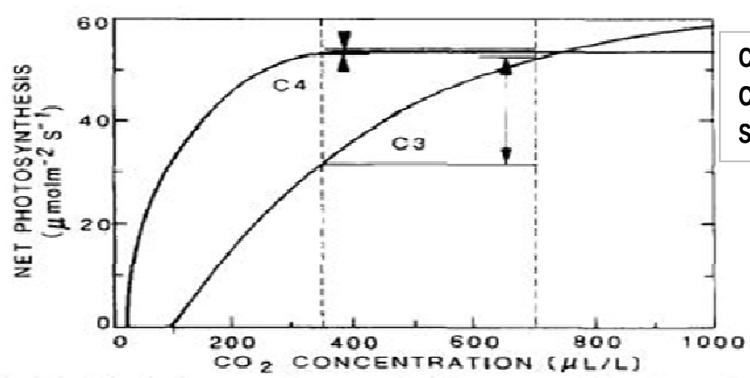
linear relationship between photosynthetic rates and photon flux densities. At higher photon flux densities, the photosynthetic response to light starts to level off and reaches saturation. Once saturation is reached, further increases in photon flux densities no longer affect photosynthetic rates, indicating other factors such as RUBISCO activity or the metabolism of triose phosphates, have become limiting.

In the Dark, CO_2 fixation is negative and there is net CO_2 evolution from the leaf because of respiration. As the photo flux density (availability of light energy) increases net CO_2 evolution decreases, after it reaches Zero, the plants shifts to net CO_2 fixation. The Photon flux density at which net CO_2 exchange in the leaf is zero is called **light compensation point**. The light compensation points of sun plants are high in the range of 10-20 u mol m^{-2} S^{-1} where as corresponding values for shade plants are low in the range of 1-5 u mol $m^{-2}S^{-1}$. The values for shade plants are lower because respiration rates in shade plants are very low, so little photosynthesis suffices to bring the rates of CO_2 evolution to zero.



LIGHT RESPONSE CURVE

11.2.2 **CARBON DIOXIDE:** Carbon dioxide is a trace gas in the atmosphere constituting approximately 390ppm.



CO_2 RESPONSE CURVES OF C_3 & C_4 SPECIES

Classical net photosynthetic curve for C_3 and C_4 species.Dashed vertical lines at 350 and 700 µ lit/lit

mark the current CO_2 level and the doubled concentration predicted to be reached some time late in the next century.Arrows indicate incremental rise in net photosynthesis due to the CO_2 doubling.

In plants with CO_2 concentrating mechanisms which include C_4 and CAM plants, the CO_2 concentrations at the carboxylation sites are often saturating. So a C_4 plant such as corn cannot increase its photosynthetic performance with the increase in atmosphere CO_2 concentrations.In the C_3 Plants, on the other hand, increasing Ci levels (Ci partial pressure of CO_2 in the inter cellular spaces of leaf) continue to stimulate photosynthesis over much broader range.

Also markedly different in C_3 and C_4 plants is the CO_2 compensation point, the CO_2 concentration at which CO_2 fixation by photosynthesis balances CO_2 loss by respiration and net CO_2 is zero. Plants with C_4 metabolism have a CO_2 compensation point of zero or nearly zero (CO_2 compensation point of C_3 plants is 35 – 50 ppm, while for C_4 plants 0 – 10 ppm.)

In C_3 plants additional CO_2 decreases photorespiration by increasing the ratio of CO_2 to O_2 ratio, which leads to faster net photosynthesis.

11.2.3 TEMPERATURE :

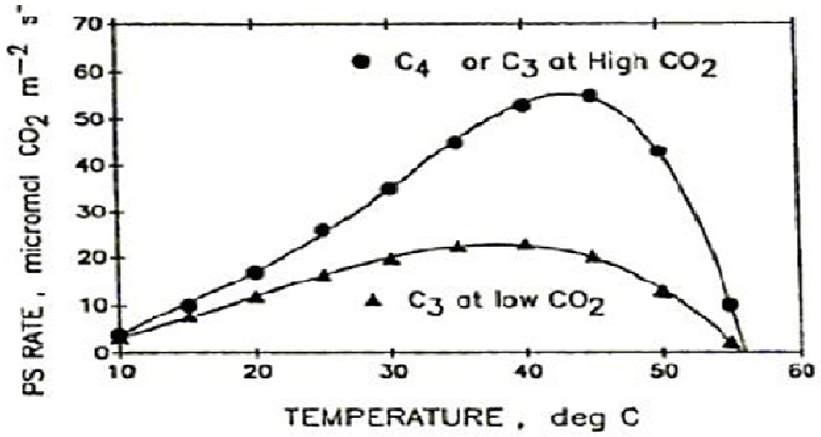
When photosynthetic rates are plotted as a function of temperature, the curves have a characteristic bell shape. The ascending arm of the curve represents a temperature dependent stimulation of photosynthesis up to an optimum, the descending arm associated with deleterious effects.

When photosynthetic rates are measured in air at normal and at high CO_2 concentrations: at high CO_2 there is ample supply of CO_2 at the carboxylation sites and the rate of photosynthesis is limited primarily by biochemical reactions. At ambient (normal) CO_2 concentrations photosynthesis is limited by the activity of RUBISCO.

Temperature rise and CO_2 fixation are conflicting processes. An increase in carboxylation rate with temperature and a decrease in the affinity of the RUBISCO for CO_2 is observed as the temperature rise. These opposite effects slow down the temperature response of Photosynthesis at ambient CO_2 concentrations.

Respiration rates increase as a function of temperature and the interaction between photorespiration and photosynthesis becomes apparent in temperature responses. The quantum yield of photosynthetic carbon fixation in a C_4 plant remains constant with temperature, reflecting typical low rates of photorespiration. In the C_3 plant, the quantum yield decreases sharply with temperature, reflecting stimulation of photorespiration by temperature and an ensuring higher energy demand per net CO_2 fixed.

Optimal temperature is the point at which the capacities of various steps of photosynthesis are optimally balanced. Although there are exceptions, C4 plants generally have higher temperature optima than C3 plants and this difference is controlled largely by lower rates of photorespiration in C4 plants.



TEMPERATURE RESPONSE CURVES

Photosynthetic rate versus temperature for C_3 and C_4 leaves.

11.2.4 Water

When water becomes limiting, cell expansion is first retarded so that growth is reduced. With only a little more water stress, stomata begin to close and CO_2 uptake is restricted. Photosynthesis is then limited by water because of retarded leaf expansion and because of restricted CO_2 absorption.

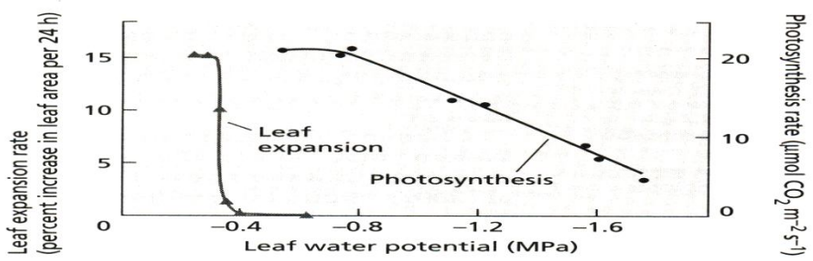


FIGURE Effects of water stress on photosynthesis and leaf expansion of sunflower (*Helianthus annuus*). This species is typical of many plants in which leaf expansion is very sensitive to water stress, and it is completely inhibited under mild stress levels that hardly affect photosynthetic rates. (After Boyer 1970.)

11.2.5 Other factor:

Factors like weedicides, salinity and water logging also affect the rate of photosynthesis. Majority of Herbicides -about half of the commercially important compounds—act by interrupting photosynthetic electron flow.(Ex. Paraquat, diuron). When the electron transport is blocked it virtually stops light reaction of photosynthesis. When light reaction is stopped the dark reaction does not happen and thus CO_2 is not fixed as carbohydrate. Therefore the weed is killed by starvation.

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Lecture-12

PHOTOSYNTHESIS

12.1 PHOTOSYNTHETIC EFFEICIENCY

Photosynthetic efficiency is the amount of dry matter fixed by the crop in a unit area per unit time .Photosynthetic efficiency (Eμ) of a crop can be estimated with the following formula

$$E\mu = \frac{\text{Chemical energy captured by a crop}}{\text{Solar energy received}}$$

Thus, photosynthetic efficiency can be understood as net gain of chemical energy per total incident solar radiation in a unit area.

Considering supplies of total PAR (Photosynthetically active radiation) to land area on a growing crop, overall biomass production efficiencies are always much below 18 percent. How ever this 18% is only a theoretical estimation which is calculated as follows. Assume that 12 moles of photons represent the maximum number of photons needed to fix one mole of CO_2 and that an average photon in the PAR region (400-700nm) has a wavelength of 550nm. From Planck's equation ($E= h\nu$) relating photon energy and wavelength we can calculate that one mole of such photons has an energy of 217,000 J (51,900 cal). Twelve moles of photons would therefore have an energy of 2.6×10^6 J. This is the input energy. The output energy, one mole of fixed carbon in carbohydrate, has energy of about 0.48×10^6 J. Efficiency equals output energy / input energy or 18 percent.

Furthermore, **only 40-45 percent of the sun's energy is in the PAR region**, so the **theoretical maximum efficiency** from all the sun's energy is only about **8 percent** (45 percent of 18 percent). However, experimentally, the efficiency of energy conversion in plants-the ratio of energy stored as organic substance to the amount of energy available from the sun varies between 0.1 to 1.0% under conventional agricultural practices to between 6 and 10% under conditions of intensive agriculture. Many crops including forest trees and herbaceous species, convert only 1 to 2 percent of the PAR striking the field during the growing season into stored carbohydrates. Much PAR is wasted by striking the bare ground between young plants before leaves have grown enough to absorb it.

Optimizing the interception of solar radiation by the canopy is an important component of biomass production. This is influenced by the rate of development of leaf area, leaf area duration and canopy architecture.

12.1.1 Photosynthetic efficiency of C_3 & C_4 crops:

In normal air, increasing temperatures gradually decrease efficiencies of C_3plants, with efficiencies of C_4plants remaining constant. As temperatures rise above 30^oC efficiencies of the most C_3plants become lower than those of C_4. This efficiency crossover with increased temperature results from lower net photosynthesis in C_3 plants because of faster CO_2 loss by photorespiration. The absence of detectable photorespiration in C_4 plants even above 30^oC gives them a substantial efficiency advantage at high but not at low temperatures and especially in non-shaded conditions

The relative photosynthetic efficiencies of C_3 &C_4 crops can be calculated by considering the energetics of these processes occurring in plants. Among C_3, C_4 and CAM pathways, it is clear that in C_3 plants occurrence of photorespiration leads to loss of Carbon through glycine decarboxylation. Further, such oxygenase function of RuBISCO increase under abiotic stress such as higher temperature, water stress etc. due to reduced levels of CO_2 in sub-stomatal cavity.

In course of evolution a relatively more efficient alternate C fixation mechanism under water limited and high temperature environments was evolved in plants i.e., "C_4 pathways" as seen in C_4 plants.

In terms of energy requirements:

- C_3 photosynthesis under normal conditions requires **3 ATP +2NADPH** for fixation of one molecule of CO_2.This is calculated from the following equation.

$$6CO_2 + 18\ ATP + 12NADPH + 12\ H^+ \longrightarrow C_6H_{12}O_6 + 18\ ADP + 18Pi + 12\ NADP + 6H_2O$$

- Under ambient CO_2 concentration / and O_2 conc. in the air, with occurrence of photorespiration simultaneously in C_3 plants this requirement increases to approximately. **5ATP + 3.2 NADPH**

On the Other hand

- In C_4 plants the energy requirement for fixation of one CO_2 molecule is **5 ATP + 2 NADPH**

$$6CO_2 + 30ATP + 12NADPH + 12H^+ \longrightarrow C_6H_{12}O_6 + 30\ ADP + 30\ Pi + 12NADP + 6\ H_2O$$

- In C_4 plants Photorespiration is negligible due to the CO_2 concentration mechanism .However, for concentrating CO_2 in bundle sheath cells it needs two additional ATPs. Thus, it needs 5 ATP.

- Any photo respiratory CO_2 evolution from bundle sheath cells is refixed by PEP carboxylase ase due to its high affinity for CO_2

Thus, on the basis of

1. Energetics in lines with C loss due to photorespiration and
2. Affinity for CO_2

It can be concluded that under ambient conditions compared to C_3 plants, C_4 are “Photosynthetically Efficient”.

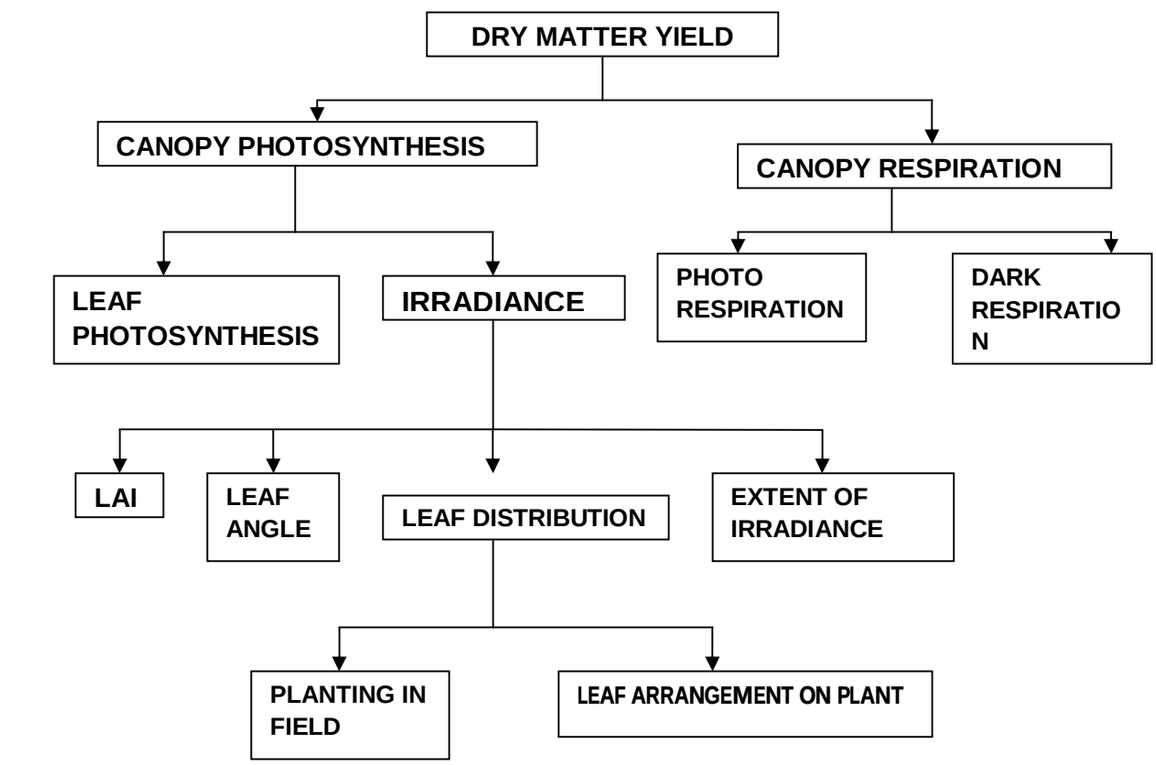
12.2 SIGNIFICANCE OF C_3, C_4 & CAM PATHWAYS- A COMPARISON

C_3	C_4	CAM
Typically temperate species e.g. rice, wheat, barley, sugar beet, soybean, sunflower, spinach, potato, tobacco, , oats and rye.	Typically tropical or semitropical species e.g. Maize, sugarcane, sorghum, Amarathus Plants adapted to high light, high temperature and also semiarid	Typically arid zone species e.g. cacti, orchids. Agave, succulent plants. Pine apple is the only cultivated crop among this group.
Moderately productive, yields of 30 tones dry weight per hectare possible (sunflower is highly productive)	Highly productive, 80t per hectare for sugarcane is possible	Usually very poorly productive (However, Pineapple is highly productive).
Mesophyll Cells containing chloroplasts do not show Kranz-type anatomy. Only one type of chloroplast.	Kranz-type anatomy is essential feature. Often have two distinct types of chloroplast (both in Budle sheath and mesophyll cells)	Lack Kranz anatomy. Only one type of Chlroplast.
Initial CO_2 acceptor is ribulose bisphosphate (RuBP) a 5 carbon sugar.	Initial CO_2 acceptor is phosphor enol pyruvate (PEP) (a 3-carbon acid)	CO_2 acceptor is PEP in the dark and RuBP in the light
Initial CO_2 fixation product is the 3 – carbon acid **phosphoglycerate**	Initial CO_2 fixation product is the 4-carbon acid **oxalo acetate.**	CO_2 fixation products are **oxalo acetate** in the dark and **phospho glycerate** in light.
Only one CO_2 fixation pathway	Two CO_2 fixation pathways separated in space.	Two CO_2 fixation pathways separated in time.
High rates of glycolate synthesis and photorespiration	Low rates of glycolate synthesis, no photorespiration.	Low rates of glycolate synthesis; no photorespiration

Low water use efficiency and low salinity (ion) tolerance	High water use efficiency and salinity tolerance.	High water use efficiency and salinity tolerance.
Photosynthesis saturates at 1/5 full sunlight	Do not readily photo saturate at highlight,	Do not readily photo saturate at high light.
High CO_2 Compensation point (50-150ppm)	Low CO_2 compensation point (0 – 10-ppm)	High affinity for $CO_2$by night
Open stomata by day (photo active)	Open stomata by day (Photoactive)	Open stomata by night ((Skoto active)

12.3 PHOTOSYNTHESIS AND CROP PRODUCTIVITY

Crop plants grow almost entirely by photosynthesis. Thus, plant productivity interms of primary production of biomass is simply a measure of the total photosynthesis of the plants less respiration, which has occurred during its growth.



Total Dry matter yield or total biomass is a consequence of crop canopy efficiency in intercepting and utilizing the solar radiation variable during the growing season. Leaves are the main plant organs which intercept solar energy. For maximum crop growth rates, sufficient leaf area must be available in the canopy to intercept most of the solar radiation incident on the crop canopy. This is called as LAI. Mere interception of solar energy is not sufficient, it has to be converted in to the biomass and it depends on the Net Assimilation Rate (NAR) Thus, total dry matter production or productivity of a crop is a factor of how long the crop can maintain an active, green leaf canopy that can produce photosynthates.

CROP GROWTH RATE = LAI X NAR

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Lecture-13

ASSIMILATE TRANSLOCATION IN HIGHER PLANTS

13.1 TRANSLOCATION OF ASSIMILATES:

The assimilate transport is a process of exchange of metabolites among the functionally specialized organs and tissues as a coordination of activities of plants as whole.

This is essentially required to know

1. The distribution of photosynthetic products in plants
2. Their accumulation in storage organs
3. Their mobilization during resumed growth and
4. The effect of climatic factors and farming practices on the above process

13.1.1 Definition

The process of sugar movement from source to sink in sieve tube is called as translocation.

13.1.2 Types of assimilate transport

The assimilate transport is of two types depending on their distance of transport.

A) **Short distance transport:** Movement of sugar from mesophyll cell to the vicinity of sieve elements in the smallest veins of the leaf involving a distance of only one or three cells diameter.

B) **Long distance transport:** Translocation of sucrose and other solutes from source to sink inside the sieve elements is referred as long distance transport.

The two pathways function simultaneously and intimately inter twined forming a continuous process encompassing the entire plant. These are extending in parallel and pressed against each other resulting in the exchange of mobile substance.

13.1.3 Anatomy of phloem tissue

Phloem is a complex tissue than the xylem. The main components of phloem are sieve tubes. These are the longitudinally arranged individual cells called sieve elements with perforated end walls called sieve plates. They are living cells. The mature sieve elements appear to contain living protoplasm, although devoid of nucleus, posses differentially permeably membrane but do not have a tonoplast. Much of the protoplasm is in the form of **P-Protein** (phloem protein) a fibrilliar protein.

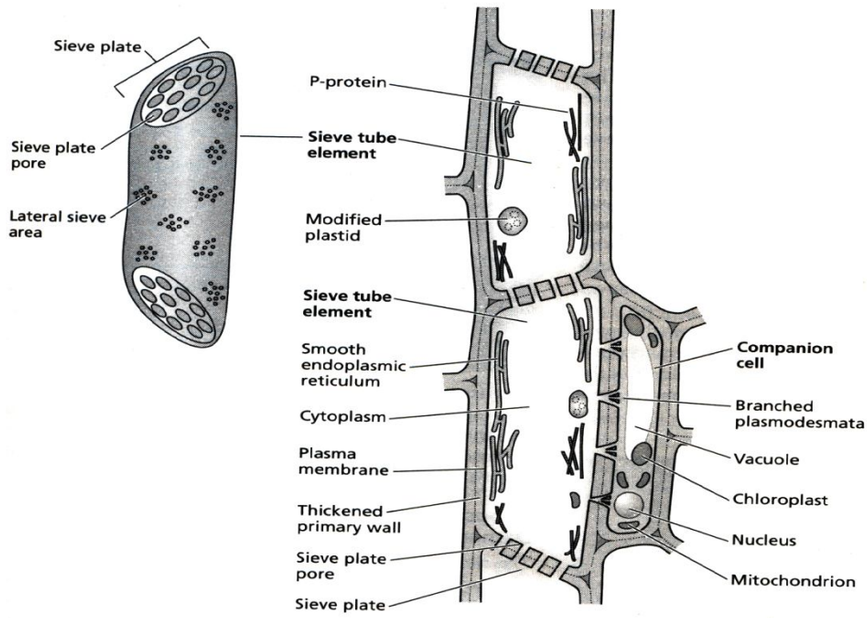


***Figure is from reference.6**

13.1.4 ASSIMILATE TRANSLOCATION IN RELATION TO "SOURCE" AND "SINK"

Assimilate partitioning involves the production of assimilates in photosynthetic organs (source) loading into sieve tubes, and translocation to the growing parts (sink), where unloading takes place. An intact plant consists of multiple "Sources" and "sink"

SOURCE

A source is any plant part that export carbon. Leaves are the principles sources of assimilates.

SINK

The centers of storage or consumption of assimilates are the "Sinks". A sink is an organ that has a net import of assimilates which would be used for growth or storage. All actively growing or metabolizing tissues are sinks.

13.2 PATHWAYS OF ASSIMILATE TRANSLOCATION

Assimilates after they are formed in the photosynthetic cells must traverse a complex short distance of 3 to 4 parenchymatous cells (tenths of a millimeter) and get concentrated in the phloem against the concentration gradient. This involves additional energy expenditure.

In this route assimilate pass across the mesophyll cells in two pathways separated or simultaneously. They are

13.2.1. Symplastic transport of assimilates (via plasmodesmata) (living part of the plant)

Along this route, the mesophyll cells are interconnected with thin strands of cytoplasm penetrating the cell walls in many places called **plasmodesmata. Munch**

(1930) termed this continuous protoplast in an aggregation of cells in complex organisms as **symplast**.

Thus, symplastic transport can be defined as Translocation of substances from one cell to another via plasmodesmata.

13.2.2 Apoplastic transport of assimilates (via free space)

In this pathway, assimilates leave the cytoplasm across its outer membrane, on to the surface of the assimilating cells, (i.e. the apoplast) where in the solution forming a film around the free intercellular space between cells.

Thus, apoplast includes all non living cells, cell walls and intercellular spaces in stems and leaves.

13.3 ASSIMILATE TRANSLOCATION CAN BE UNDERSTOOD FROM THE FOLLOWING HEADINGS

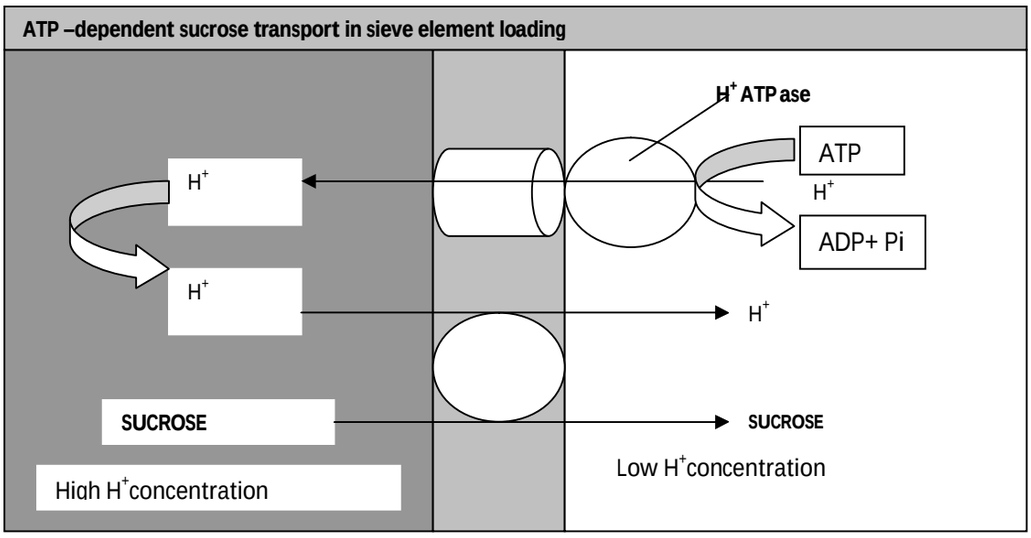
- Phloem loading
- Mechanism of phloem transport
- Phloem unloading

13.3.1 <u>PHOLOEM LOADING</u>

The site of phloem loading is the **sieve cell- companion cell complex** of source. In source leaves, sugars become more concentrated in the sieve elements and companion cells than in the mesophyll cells. So in phloem loading the sucrose is transported against its chemical potential gradient and is evidence for active transport of this solute.

Sugars might move entirely through the symplast via the plasmodesmata or, alternatively might enter the apoplast at some point enroute to the phloem. In the later case, the sugars could be actively loaded from the apoplast into the sieve elements and companion cells by an energy driven carrier located in the plasma membranes of these cells.

So the uphill transport of sucrose from the apoplast into the sieve cells requires energy in the term of ATP. **Giaquinta** (1977) proposed **sucrose proton transport model** involving energy. According to this an ATP ase builds up a proton gradient across the plasma membrane by splitting ATP into ADP + Pi and excreting the resulting H^+ into the apoplast. The sucrose is taken up by sucrose proton and is transported across membrane mediated by a carrier which is activated by protonation. High ATP ase activity at the surface of the sieve element cell justified this assumption.



13.3.2 MECHANISM OF PHLOEM TRANSPORT (Munch pressure Flow Hypothesis)

Munch pressure flow system is the only one understandable mechanism for translocation of assimilates in plants. The workable hypothesis of Munch system is mass flow or pressure flow. It is driven by metabolic processes of sources and sink tissues. Phloem loading by the sources tissue exerts **push** and unloading by the sink tissue **pull,** both driving the mass flow in the phloem. A sequence of physiological steps occur in this system are shown in the following figure.

The assimilates from the mesophyll cells of source when actively loading up in phloem, water potential of the phloem sap decreases. Consequently water is drawn from xylem tissues into the sieve cells raising the hydrostatic pressure. This pushes assimilates to flow enmasse, from one sieve tube to the other uninterruptedly through open sieve pores. About 70% of sieve plate pores are freely kept open in a number of other angiosperm species. Only on wounding **callose**, a carbohydrate is synthesized which plugs sieve pores to protect from a major loss of photosynthates.

In the physiological sink, the assimilates are removed from the sieve cells thus the water potential increased and water moves out of sieve cells into xylem. Both osmotic water up take in the sieve cells of the source and water loss from the sieve cells of sink, result in the circulation of water under pressure flow from the source to the sink (translocation) in the phloem carrying assimilates, down to the sink cell and water from sink cells to source cells (Transpiration) in the xylem.

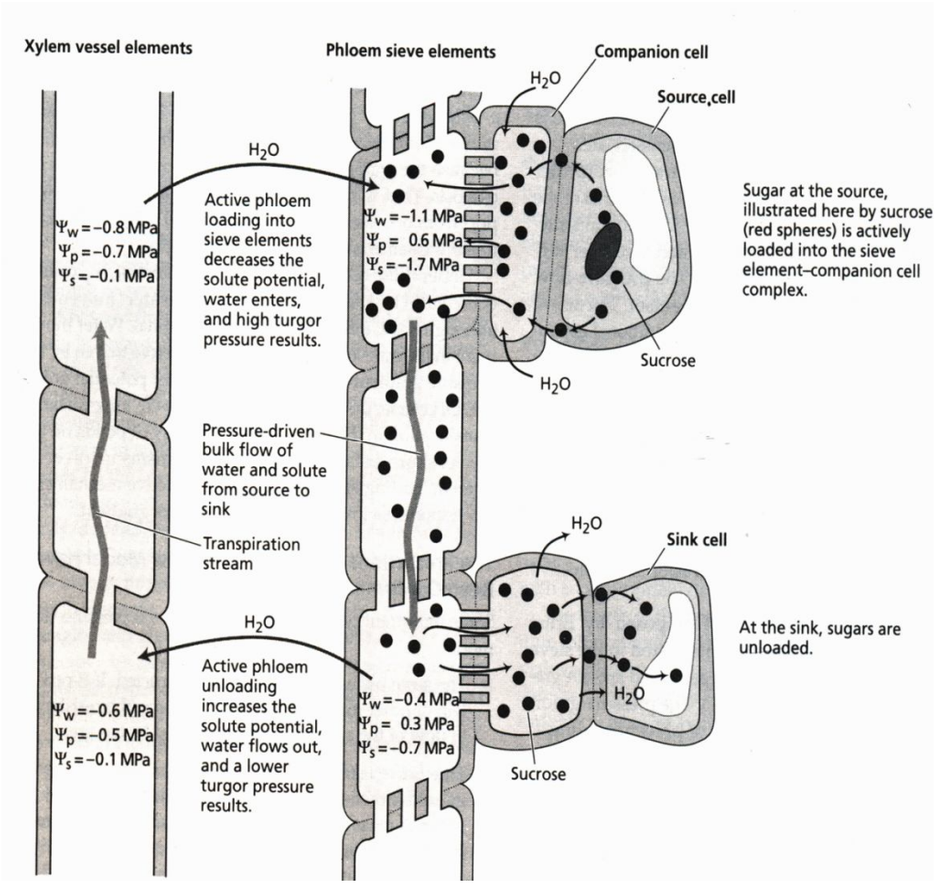


***Figure is from reference.6**

Composition of phloem sap:

Sucrose is the most important mobile organic solute (200-300 mM) followed by amino acids (30-200mM) and organic acids. Among inorganic solutes it is potassium that dominates.

13.3.3 PHLOEM UNLOADING:

The process occurs at the site of sink. In many ways the events in sink tissues are simply the reverse of the events in sources. Transport in to sink organs, such as developing roots, tubers and reproductive structures, is termed import. The following steps are involved in the import of sugars in to sink cells.

1. Sieve element unloading: This is the process by which imported sugars leave the sieve elements of sink tissues
2. Short distance transport: After sieve element unloading, the sugars are transported to cells in the sink by means of a short distance transport pathway. This pathway has also been called *post sieve element transport*.
3. Storage and metabolism: In the final step, sugars are stored or metabolized in sink cells.

This three transport steps together constitute phloem unloading, the movement of photosynthates from the sieve elements and their distribution to the sink cells that store or metabolize them.

PHOLEM UNLOADING IS BOTH SYMPLASTIC AND APOPLASTIC:

In vegetative sinks that are growing such as roots and young leaves, phloem unloading and transport into receiver cells are usually symplastic. In other sinks unloading is apoplastic.

Ex. Apoplastic unloading is required in developing seeds because there are no symplastic connections between the maternal tissues and the tissues of the embryo.

When unloading is symplastic, transport of sugars occurs through plasmodesmata to the receiver cells where it can be metabolized in the cytosol vacuole i.e., Passive unloading occurs from a high concentration sieve elements to low concentration in sink cell.

APOPLASTIC TRANSPORT IS ENERGY DEPENDENT:

When unloading is apoplastic, the transported sugars can partially be metabolized in the apoplast (free space) it self. Ex. In sugarcane sucrose splits into glucose and fructose by an enzyme invertase in the apoplast itself and glucose or fructose that is taken up by the receiver cell.

In case of apoplastic unloading, sugars must cross at least two membranes

1. Membrane of sieve element and companion complex
2. Membrane of the receiver cell.

This transport of sugars across the membrane is shown to be active Thus apoplastic transport is an energy dependent process. Phloem unloading is strictly controlled by sink metabolism. Faster the metabolism greater will be the sink demand.

It also depends upon the activity of phytohormones .In case of legumes, number of nodules and nodule activity also controls phloem unloading.

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Lecture-14 **SOURCE AND SINK CONCEPT**

14.1 SOURCE AND SINK CONCEPT

Determination of factors that influence yield is a large and intensively studied area of crop physiology. Yield is the manifestation of all physiological processes occurring in plants. There are different types of yield among the domesticated crops. They are as follows.

Crop	**Types of yield / Storage organ**
Cereals	Seed / grains mainly for CH2OS
Pulses	Proteins
Oilseeds	Seed mainly for lipids / oils / waxes
Sugarcane	Cane for sugar
Fibers	Bolls for lint especially in cotton
Tubers	Tubers for CH_2Os
Fodders	Fresh or dry weight of stalk

Since yield is the resultant of dry matter partitioned between the different parts of plant, possibilities of changing the distribution of assimilates in crops by physiological manipulation is one of the most promising ways of increasing agricultural productivity. To achieve this, knowledge of source – sink concept is important to understand the direction of transport of assimilates.

The movement of assimilates from source to sink is currently believed to occur as follows

1) The photosynthetic source cell produces the sugars, which can move symplastically or apoplastically to the sieve tubes.
2) Phloem loading increases the sugar concentration of sieve tubes above that of the apoplast.
3) At the sink, carbohydrates are being absorbed and either actively partitioned into cell constituents (eg. Starch) or changed to other carbohydrates. Phloem unloading lowers the concentration of sugars in sieve tubes.
4) The buildup of sugars at the source and the removal of sugar at the sink establish a hydrostatic pressure gradient, which moves water and sugar from sources to sinks.

Thus, higher production of assimilates (source strength), their rapid translocation and utilization in growth and development (sink strength) are some key factors of concern to increase crop yield.

14.2 DRY MATTER PARTITIONING IS DECIDED BY SOURCE STRENGTH AND SINK STRENGTH:

14.2.1 SOURCE STRENGTH:

Source strength is a function of source size and source activity.

Source strength = Source size X source activity

The total amount of photosynthates produced in a crop is decided by the optimum crop canopy size (actively photosynthesizing leaf area or source size) and the rate of assimilation per leaf area (photosynthetic efficiency or source activity) of crops. The estimates of efficiency of utilization of photo synthetically active radiation (PAR) some crops is given below.

Crop	**Utilization of PAR (%)**
Maize (*Zea mays*)	6.2
Sugarcane (*Saccharum spp*)	7.7
Rice (*Oryza sativa*)	6.2
Sugar beet (*Beta vulgaris*)	8.8
Soybean (*Glycine max*)	7.7

14.2.2 SINK STRENGTH:

Sink strength is a function of sink size and sink activity.

Sink Strength= sink size X sink activity

Sink size or sink capacity is the maximum space available for accumulation of photosynthetic products.In grain crops it is expressed as number and size of grains. **Sink activity** is the inherent capacity of the sink to create a translocation gradient for photosynthates assimilated at source.

According to the mass flow hypothesis, any increase in photosynthesis increases hydrostatic pressure and translocation rate. However, this is true only if sinks have the ability to utilize more assimilates. If they are unable to utilize the increased production there would be a steady build up of sugars in the system causing a **feedback inhibition** resulting in reduced photosynthesis. Presumably, photosynthetic rate would be altered to the rate at which sinks could accept assimilates.

Thus, for leaf photosynthesis to be at maximum potential rates, sinks must be able to rapidly utilize the assimilates produced by the source. Under these conditions partitioning would be controlled by sink strength that is sink availability and the rate at which available sinks can utilize assimilate (sink demand).

Role Of Hormones In Alteration Of Source-Sink Relationship: The effect of hormones on enzymatic activity and the elasticity of sink cells can have a dramatic effect on partitioning. Indoleacetic (IAA), cytokinins, ethylene and gibberllic acid, when applied to cut stem surfaces causes assimilates to accumulate in the region of application In bean seedlings, the main control over the distribution of sucrose between root and shoot sinks can be attributed to auxin and cytokinin concentrations in various sinks. Hormonal influences on initiation, development and abortion of flowers and seeds have a significant effect on the source – sink relationships in crops.

14.3 HARVEST INDEX

Two useful terms used to describe partitioning of dry matter by the plant are **Biological yield & economic yield**. The term biological yield was proposed by **Nichiporovich** (1960) to represent the total dry matter accumulation of a plant's system. Economic yield and agricultural yield have been used to refer to the volume or weight of those plant organs that constitute the product of economic or agricultural value. The proportion of biological yield represented by economic yield has been called the harvest index or the migration coefficient. These terms characterize the movement of dry matter to the harvested part of the plant. The harvest index, the most widely used tem, is defined as follows.

$$\text{Harvest index} = \frac{\text{Economic yield}}{\text{Biological yield}} \times 100$$

(It must be remembered that the biological yield total, often does not include root weight because of the difficulty in obtaining those values)

Crop yield can be increased either by increasing the total dry matter produced in the filed or by increasing the proportion of economic yield (the harvest index) or both. There is potential for increasing yields by both methods.

The economic fraction of biological yield usually referred to harvest index varies among the species.

Crop	**Harvest Index (%)**
Cereals	50 – 60
Pulses	30 – 45
Oilseeds	45 – 50
Sugarcane	35

Yield of a crop is a function of biomass X Harvest index .Grain yield in cereals is a product of dry matter accumulation and partitioning.

Thus, grain yield can be understood as product of a number of subtractions called yield components which can be expressed as follows.

Y = Nr Ng Wg

Where, Y = grain yield

N = number of reproductive units (e.g heads, ears, panicles per unit of ground areas)

Ng= the number of grains per reproductive units and

Wg- the average weight per grain

Thus, the economic yield (Harvest index) depends upon the total dry matter produced and the amount of which is partitioned towards reproductive units in the post anthesis period. Yield components are affected by management, genotype and environment.

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Lecture-15

RESPIRATION

15.1 INTRODUCTION

The word respiration is derived from the Latin word '*respirare'* (literally, to breathe). Aerobic (oxygen requiring) respiration is common to nearly all eukaryotic organisms. It is a biological process by which reduced organic compounds are mobilized and subsequently oxidized in a controlled manner. During respiration,free energy is released and transiently stored in a compound, ATP, which can be readily utilized for the maintenance and development of plants.

Respiration can be defined as '**an oxidation-reduction process**' in which respirable substrates such as Carbohydrates, proteins, fats etc. are oxidized to CO_2 and O_2 absorbed is reduced to water, with the release of chemical energy stored in the bonds of the complex molecules (ATP).

The overall respiration process may be represented as

$$C_6H_{12}O_6 + 6\ O_2 \longrightarrow 6CO_2 + 6H_2O + 686\ K.Cal.$$

Mitochondria and respiration:

Various enzymes of oxidation of pyruvic acid (TCA Cycle) and electron transport chain (which is a series of oxidation-reduction reactions) are located in matrix of Mitochondria .The mitochondrion is made of double unit membrane. The inner layer of this membrane is deeply folded inward to form the cristae (transverse membranes) that lie more or less cross wise in the mitochondrion. The membrances appear to have small knob like structures about 70 A^0 in diameter called F1 – ATP ase attached to their inner surface by stalks about 30 A^0 long called F_o particles. These structures are concerned with the synthesis of ATP, the cells energy mobilization compound.

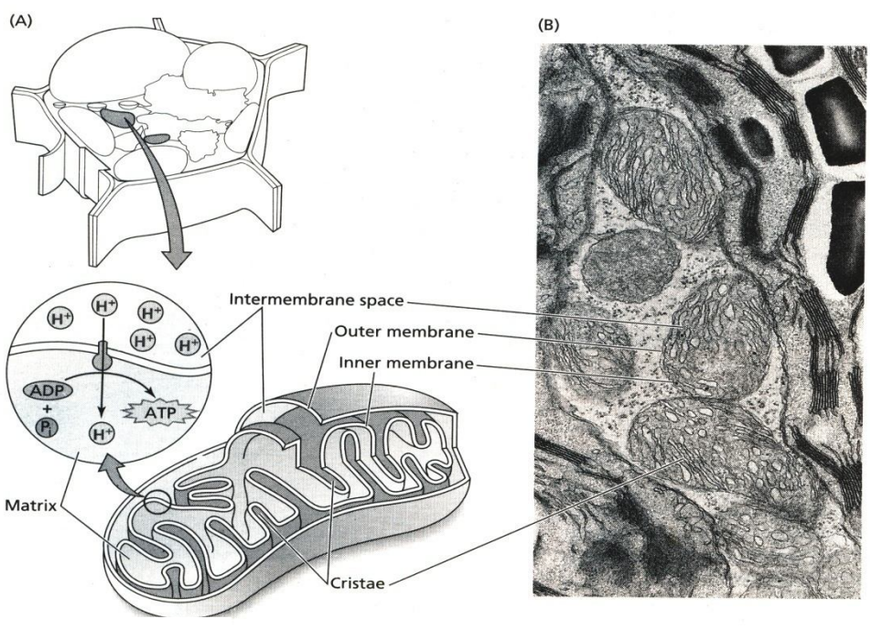


*Figure. is from reference 6

15.2 SIGNIFICANCE OF RESPIRATION:

Respiration is an important process because

1. It releases energy which is consumed in various metabolic processes essential for plant life and activates cell division.
2. It brings about the formation of other necessary compounds participating as important cell constituents.
3. It converts insoluble food into soluble form.
4. It liberates CO_2 and plays a part actively in maintaining the balance of carbon cycle in nature.
5. It converts stored energy (potential energy) into usable form (kinetic energy).

Respiration is complex process which includes:

i. Absorption of oxygen.
ii. Conversion of carbohydrate (complex) to CO_2 and water (simpler substances) i.e., oxidation of food.
iii. Release of energy.
iv. Formation of intermediate products playing different roles in metabolism
v. Loss of weight in plants as a result of oxidation.

15.3 Out line of Respiratory Metabolism:

The breakdown of a respiratory substrate in respiration, proceed through a series of reactions each catalyzed by a specific enzyme. The sequence of reactions is generally referred to as respiratory metabolism. Which Includes the following steps.

STEP I : Glycolysis:

This step operates in the cytoplasm and is common to both aerobic and anaerobic respiration. Pyruvic acid ($CH_3COCOOH$) – a key respiratory metabolite – is formed from **oxidation of glucose** (starting point for respiratory metabolism in plants) in a series of reactions collectively called as Glycolysis.

Oxidative Pentose Phosphate Pathway

It is another sequence of reactions where in glucose molecule after its phosphorylation as Glucose – 6 – Phosphate is directly oxidized to phosphogluconic acid and then to ribulose – 5 – phosphate. Electrons released in between these two steps reduce NADP to $NADPH_2$, each molecule of which after passing through ETS system produces 3 molecules of ATP like in glycolysis. This is another way of breakdown of glucose and production of energy.

STEP-II: Tricarboxylic Acid Cycle (TCA cycle/Krebs Cycle/ Citric Acid Cycle)

Pyruvic acid produced by glycolysis is oxidized in a sequence of reactions referred to as tricarboxylic acid cycle. Several intermediates of the tri carboxylic acid cycle under go decarboxylations so that CO_2 is produced. Other

intermediates are oxidized through the agency of the co – enzyme NAD^+ and hence NADH (reducing power) is generated in the process.

STEP III: Respiratory chain / Electron Transport Chain

NADH is oxidized via, the respiratory chain, so that NAD^+ is regenerated. Electron Transport Chain transfers electrons from NADH – produced during glycolysis, the pentose phosphate pathway and the TCA cycle – to Oxygen. **O_2 is the final electron acceptor** in the respiratory chain and is reduced to water. This electron transfer releases a large amount of free energy much of which is conserved through the synthesis of ATP from ADP and Pi catalyzed by the enzyme ATP Synthase. Collectively the redox reactions of the electron transport chain and the synthesis of ATP are called **Oxidative Phosphorylation**. (synthesis of ATP in light reaction of photosynthesis is called as photophosphorylation).

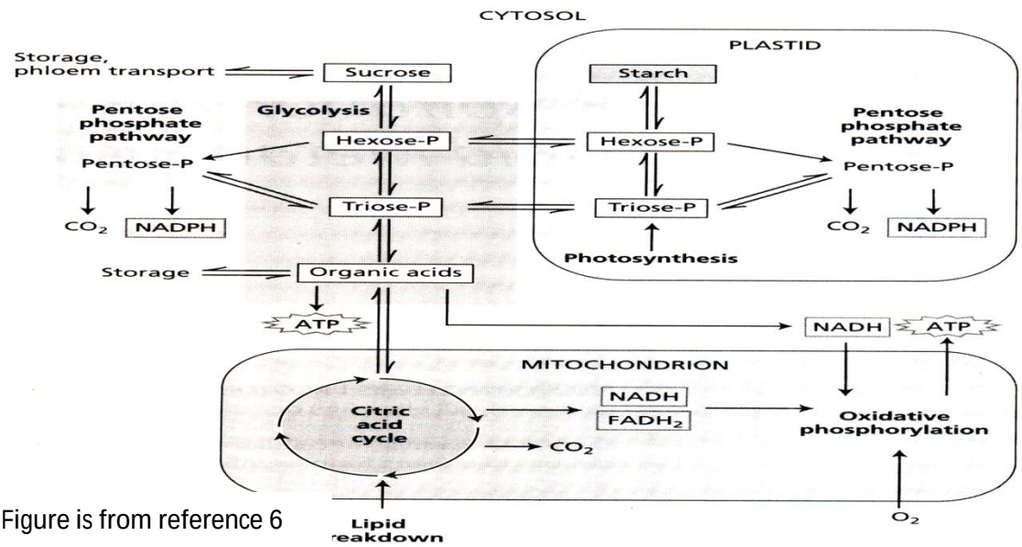
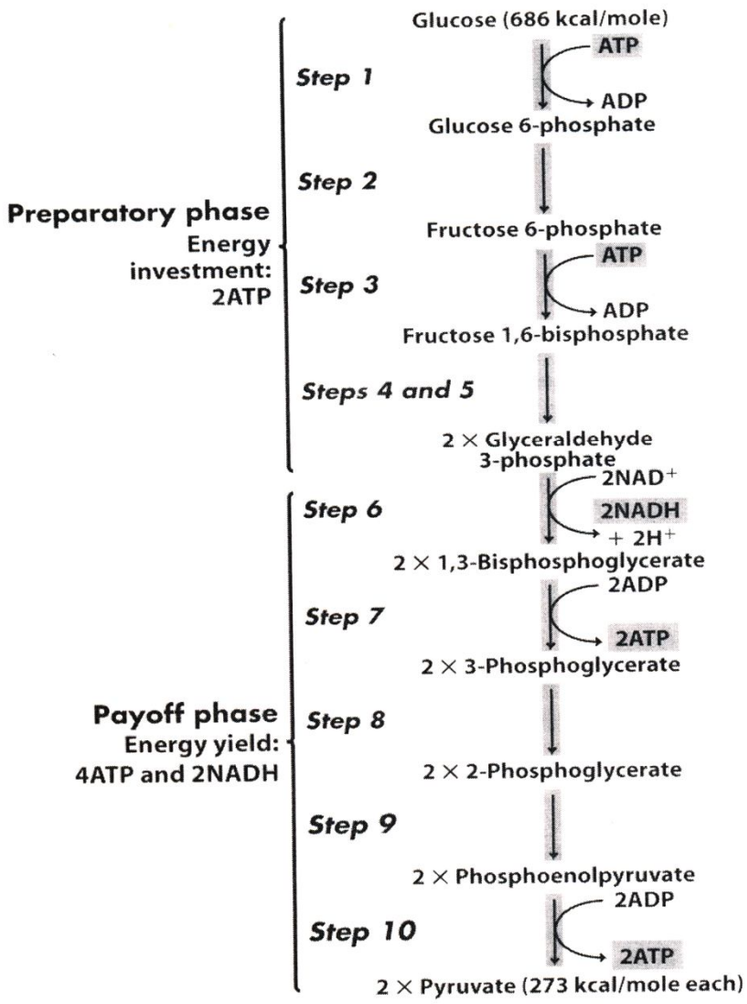


*Figure is from reference 6

15.4 <u>GLYCOLYSIS:</u>

The reactions of glycolysis process may be divided into two phases: **Preparatory phase** and **Oxidative phase**. Preparatory phase consists of 4 steps and oxidative phase of 5 steps. During preparatory phase glucose is converted into fructose – 1,6 diphosphate and during oxidative phase fructose 1,6 diphosphate split into two three carbon compounds which are converted to **pyruvic acid**.

Glycolysis



REACTIONS OF GLYCOLYSIS

Preparatory phase

In this step glucose is phosphorylated with ATP in the presence of the enzyme hexokinase to produce glucose-6- phosphate and ADP. Then glucose 6 Phosphate is converted to its isomer fructose-6- Phosphate in the presence of phospho gluco isomerase enzyme. Fructose 6 phosphate in the presence of ATP molecule and the enzyme phopho hexokinase forms fructose 1-6 diphosphate and ADP.

Oxidative phase

In this step splitting of fructose 1,6 – diphosphate into two three – carbon compounds – 3 phopho glyceraldehyde and dihydroxy acetone phosphate in the presence of enzyme **aldolase** occurs. These two sugars are inter converted through the enzyme phopho triose – isomerase.

If 3 – Phosphoglycerladehyde is depleted, additional amounts will be formed by the isomerization of dihydroxyacetone phosphate, continuing with glycolysis, 3-phosphoglyceraldehyde is converted to *1,3 – diphosphoglycerate.* This reaction involves the incorporation or addition of inorganic phosphate to first carbon of 3 phosphoglyceraldehyde and the reduction of NAD^+ to NADH and is catalyzed by the enzyme *phosphoglyceraldehyde dehydrogenase.*

The consumption of incorgnic phosphate in the oxidation of 3 – phosphoglyceraldehyde is important to the plant because this phosphate is involved in the synthesis of ATP in the next reaction of the glycolytic sequence. In the presence of ADP and the enzyme *phosphoglycero kinase,* 1,3-diphosphoglycerate is converted to *3 - phospho-glycerate* and ATP. The process of ATP formation by the transfer of phosphate from one of the intermediates of the pathway (in this case; 1,3 – diphosphoglycerate) to ADP is termed as ***substrate level phosphorylation*** . This process represents the major way in which ATP is generated from bond energy under anaerobic conditions and is particularly important to fermentation.

The 3 – Phosphoglycerate that is formed in the above reaction is transformed to 2 – *phosphoglycerate* by the activity of the enzyme *Phosphoglyceromutase.* The elimination of the elements of water (dehydration) from 2 – Phosphoglycerate in the presence of enolase results in the formation of *phosphoenolpyruvate.* In the presence of ADP and pyruvate kinase, phosphoenolpyruvate is transferred to ADP to form ATP, another example of substrate level Phosphorylation,

5.4.1 Significance of Glycolysis:

The EMP pathway(or glycolysis) , which is also referred to as the *hexose diphosphate pathway,* is the chief pathway in which glucose or intermediates are converted to pyruvate (Pyruvic acid). It involves the inter conversion of sugars and transfer of phosphate groups and the ultimate conversion of one six – carbon compound to two three – carbon molecules. It is an anaerobic pathway in which some NADH and ATP is generated. The ATP is generated by substrate level phosphorylation. The overall reaction sequence for the EMP pathway is based on the following:-

Overall Reaction Sequence

$$1\ \text{Glucose} + 4\ \text{ADP} + 2\ \text{ATP} + 2\ \text{Pi} + 2\text{NAD} \longrightarrow 2\ \text{Pyruvate} + 2\ \text{ADP} + 4\ \text{ATP} + 2\ \text{NADH}$$

Balanced Reaction

$$1\ \text{Glucose} + 2\ \text{ADP} + 2\text{Pi} + 2\text{NAD} \longrightarrow 2\ \text{Pyruvate} + 2\ \text{ATP} + 2\ \text{NADH}$$

In the first phase, the conversion of glucose to fructose – 1,6 – diphosphate, there is no energy gain. Indeed, two ATP molecules are consumed for every glucose molecule phosphorylated. However, in the second phase, the conversion of fructose – 1,6 – diphosphate to two molecules of pyruvate, four ATP molecules are formed – two for each triose split off from fructose – 1,6 – diphosphate. If we consider the complete EMP pathway, the conversion of one molecule of glucose to two molecules of pyruvate results in a net gain of two ATP and two NADH molecules.

In the sequence of reactions of glycolysis there is a net gain of two molecules of ATP. These steps may be summarized as follows:

Glucose + 2ATP ⟶ Fructose 1,6-diphosphate + 2ADP

2 ATP molecules are used

2(1,3 – DiPhosphoglyceric acid) + 2ADP ⟶ (3- phosphoglyceric acid) +2 ATP

2 (2- phosphoenolpyruvic acid) + 2ADP ⟶ 2 Puruvic acid + 2ATP

4 ATP molecules produced

(Since 2 ATP are used, there is a net gain of 2 ATP only)

However, Two $NADH_2$ molecules are also produced in the process and from its each molecule 3 ATP molecules are produced. As a result 6 more ATP molecules are formed, though these are from ETS chain. In eukaryotes, $NADH_2$ from Glycolysis enters one step late in ETS (due to loss of energy or utilization of one ATP in transfer), hence it yields only 2 ATP molecules, but in prokaryotes, it yields 3 ATP molecules.

15.5 FATE OF PYRUVATE TO CO_2 AND H_2O: (Aerobic Respiration)

Breakdown of Pyruvate is of considerable importance and significance from the stand point of energy production. In the absence of oxygen it may be degraded to Ethyl alcohol and CO_2 or Lactic acid or Alanine but in presence of Oxygen the usual fate of Pyruvate is to degrade up to CO_2 and H_2O level through Krebs Cycle and respiratory chain in which energy is trapped in the form of ATP molecules. Mitochondria have all the enzymes necessary for complete oxidation of pyruvic acid to CO_2 and H_2O. All enzymes of TCA cycle are found in the mitochondrial matrix. A summary representation of the scheme can be **Krebs Cycle**

Pyruvate ⟶ CO_2 + electrons + Protons

Respiratory Chain (electron transport chain)

Pi + ADP + electrons + protons + Oxygen ⟶ Water + ATP

15.6 FORMATION OF ACETYL COA

Pyruvic acid cannot directly enter in to the krebs cycle and therefore converts in to Acetyl Co-A. The Oxidative decarboxylation of Pyruavte into Acetyl CoA involves the presence of at least five essential co factors and a complex enzyme. The Cofactors involved are Mg ions, thiamine pyrophosphate (TPP), NAD^+, Co enzyme A (CoA) and Lipoic Acid.

15.7 KREBS CYCLE (CITRIC ACID CYCLE, TRICARBOXYLIC ACID CYCLE)

The first reaction of the krebs cycle is the condensation of acetyl CoA with oxaloacetate to form citric acid and release CoA. The result of this reaction, catalyzed by condensing enzyme, is that a four carbon dicarboxylic acid is converted to a six carbon tri carboxylic acid.(Fig.)

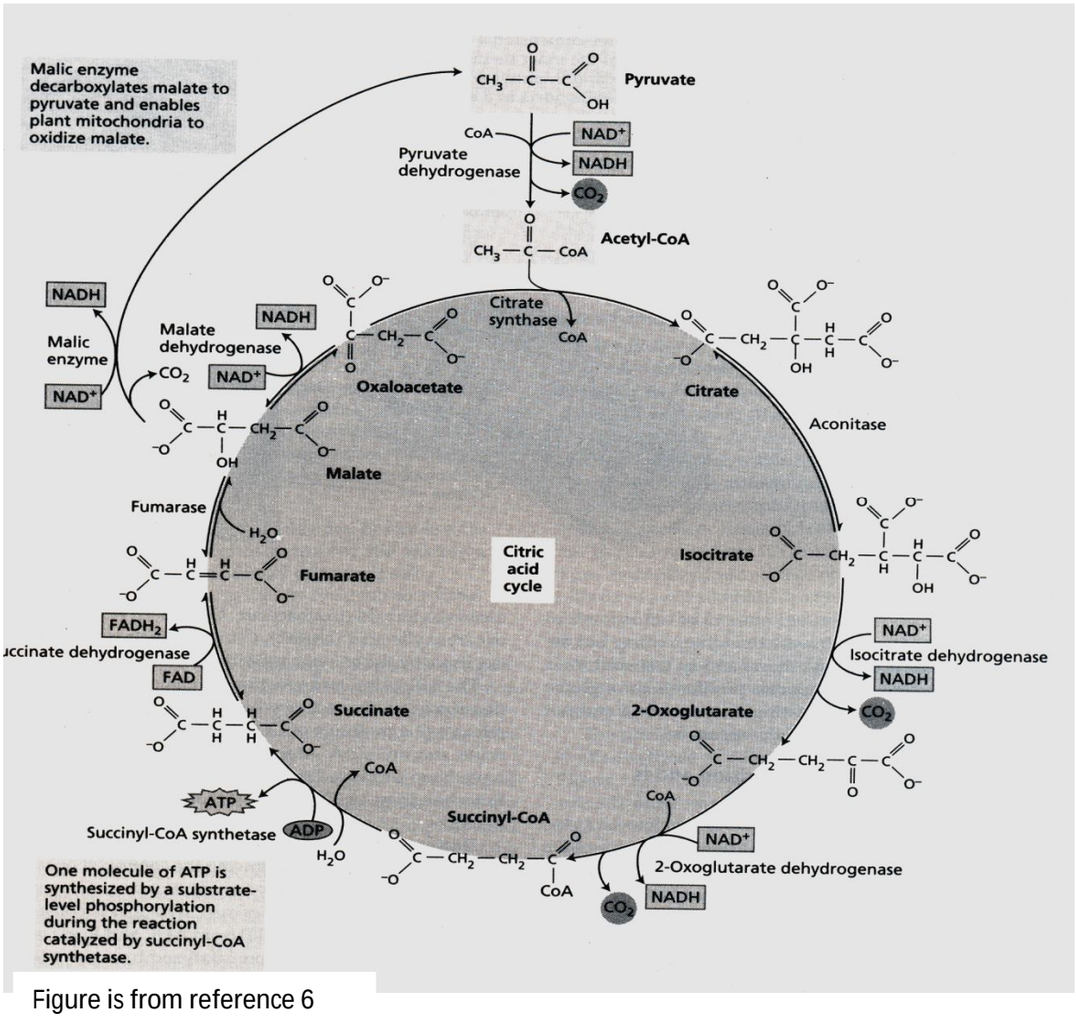


Figure is from reference 6

Through a series of reactions involving four oxidation steps and three molecules of H_2O (one utilized in the condensation reaction), oxaloacetic acid is regenerated from citric acid. In the process two molecules of CO_2 and 8 H atoms are released. We should note that these acids and other organic acids of the Krebs cycle are displayed in the ionized form ($R - COO^-$) and are labeled accordingly (i.e., Citrate, oxaloacetate, and so on.)

Acetyl CoA is the connecting link between glycolysis , and the Krebs Cycle (*citric acid cycle or tricarboxylic acid cycle)* so named because of the cyclic manner in which the starting compound, *oxaloacetate* is regenerated. The cycle is named after an English biochemist Krebs who played a major role in its discovery.

15.8 SIGNIFICANCE OF TCA CYCLE:

In the four oxidation steps four pairs of H^+ ions and four pairs of electrons are removed from the intermediates of the cycle. Three of these pairs of H^+ ions and electrons are utilized in the reduction of pyridine nucleotides (NAD). The one remaining pair of H^+ ions and electrons is taken up in the reduction of the FAD prosthetic group of succinic dehydrogenase.

The NADH and FADH produced by the reactions of the Krebs cycle represent reducing power that can be harnessed in the presence of oxygen to produce ATP. The production of ATP is accomplished by a series of oxidation-reduction reactions involving chemicals that comprise the electron transport system (ETS). The ETS is intimately associated with the mitochondrial membranes and the Krebs cycle apparatus.

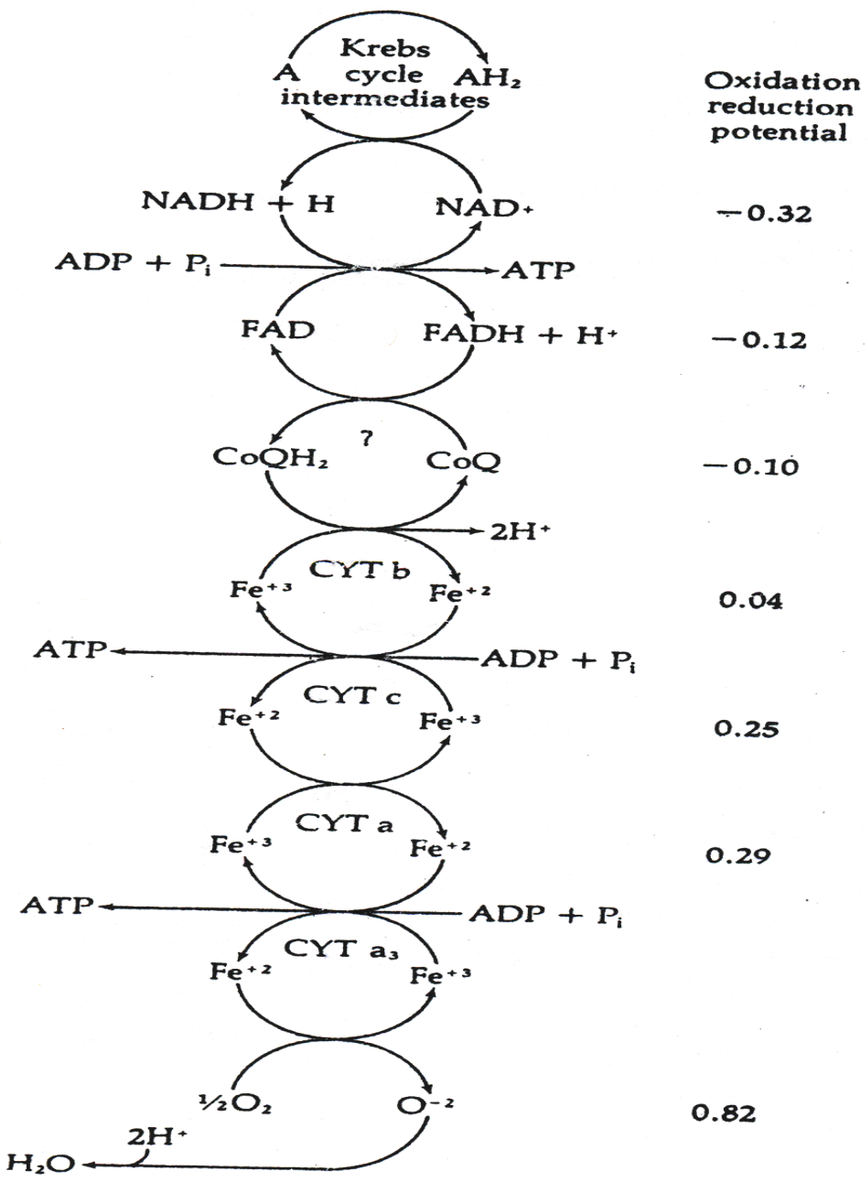
By means of the Krebs cycle, and the *electron transport system,* pyruvate is oxidized to CO_2 and H_2O. Thus the complete oxidation of glucose to CO_2 and H_2O may occur through glycolysis, the Krebs Cycle, and the electron transport system. Through its association with the electron transport system, the oxidations of the krebs cycle can account for the formation of 24 ATP molecules. Thus the krebs cycle is far more efficient in the release of energy than are either glycolysis or fermentation. The reactions of the krebs cycle and the electron transport system require the presence of oxygen and are confined to the mitochondria.

15.9 ELECTRON TRANSPORT SYSTEM AND PHOSPHORYLATION:

For aerobic organisms it is essential that the enzymes and reduction products of the Krebs cycle be associated with the Electron Transport System. Through this association, the reduced pyrimidine nucleotide NADH, FADH, and (rarely) NADPH are reoxidized. The energy released from these oxidations is utilized in the synthesis of ATP. The synthesis of ATP via electron flow through the ETS, with oxygen as the terminal electron acceptor, is known as *oxidative phosphorylation* and takes place in mitochondria.

In essence, the ETS is a chain of carriers consisting of nicotinamide adenine dinucleotide (NAD) flavin nucleotides (FAD, sometimes FMN,) Co enzyme Q (CoQ) , and the cytochromes (cyt b, c, a, a_3) and non-heme iron proteins also seem to be involved but their role is not known exactly.

Most important to the living plant is the fact that each step in the system is characterized by a decrease in the energy level. In other words, the carriers presumably operate in order of an increasing tendency to undergo reduction (reducing potential becomes increasingly positive from NADH through cytochrome a_3.



The electron will flow from a higher to lower energy level. Thus with each step in the system the energy level of the electron is lowered and the energy difference is transformed into phosphate bond energy by the conversion of ADP to ATP. Hydrogen ions are released in the oxidation of reduced co enzyme Q (CoQ).

These hydrogen ions may play an important role in ATP production. Only the electrons are passed along the series of cytochromes.

15.9 SIGNIFICANCE OF ELECTRON TRANSPORT CHAIN:

A further study will show that for every pair of electrons passed along this system, three ATP are formed. The synthesis of ATP occurs in the oxidation of NADH, in the oxidation two cytochrome b's and in the oxidation of two cytochrome a's. At their lowest energy level, the electrons are passed to oxygen from reduced cytochrome a_3 thereby activating the oxygen. In this state, oxygen will accept free hydrogen ions to form water.

15.10 ENERGY BALANCE SHEET OF RESPIRATORY METABOLISM:

If we now consider the complete degradation of a molecule of glucose, first to two pyruvic acid molecules via the EMP pathway and then to the two acetyl CoA molecules through the oxygen-requiring Krebs cycle to CO_2 and water, it is possible to have thirty-eight ATP molecules generated.

Pathway	NADH (3ATP)	FADH (2ATP)	ATP	Total ATP
EMP (yield)	2 (6)	0	2	8
Pyruvic acid to Acetyl CoA	2 (6)	0	0	6
Krebs Cycle (yield)	6 (18)	2 (4)	2	24
Total ATP	10 X 3 = 30	2X 2 = 4	4	38 ATP

15.11 THE PENTOSE PHOSPHATE PATHWAY PRODUCES NADPH AND BIOSYNTHETIC INTERMEDIATES:

The glycolytic pathway is not the only route available for the oxidation of sugars in plant cells. Sharing common metabolites, the **oxidative pentose phosphate pathway** (also known as the ***hexose monophosphate shunt)*** can also accomplish this task. The reactions are carried out by soluble enzymes present in the cytosol and in plastids. Generally, the pathway in plastids predominates over the cytosolic pathway.

The First two reactions of this pathway involve the Oxidative events that convert the six – carbon glucose – 6 – phosphate to a five – carbon sugar, ribulose 5 – phosphate, with loss of a CO_2 molecule and generation of two molecules of NADPH (not NADH). The remaining reactions of the pathway convert ribulose 5 phosphate to the glycolytic intermediates glyceraldehyde,3- phosphate and fructose-6- phosphate. Because glucose 6 phosphate can be regenerated from

glyceraldehyde- 3- phosphate and fructose- 6- phosphate by glycolytic enzymes, for six turns of the cycle we can write the reactions as follows:

$$6\ \text{Glucose-6-P} + 12\text{NADP}^+ + 7\text{H}_2\text{O} \longrightarrow 5\ \text{Glucose-6-P} + 6\ \text{CO}_2 + \text{P}_i + 12\text{NADPH} + 12\text{H}^+$$

The net result is the complete oxidation of one glucose – 6- phosphate molecule to CO_2 with the concomitant synthesis of 12 NADPH molecules.

Studies of the release of $^{14}CO_2$ from isotopic ally labeled glucose indicate that glycolysis is the moiré dominant breakdown pathway, accounting for 80 to 95% of the total carbon flux in most plant tissues. However the pentose phosphate pathway does contribute to the flux and developmental studies indicate that its contribution increases as plant cells develop from a meristematic to more differentiated state.

The oxidative pentose phosphate pathway plays several roles in plant metabolism:

- The Product of two oxidative steps is NADPH, and this NADPH is thought to drive reductive steps associated with various biosynthetic reactions that occur in the cytosol such as **lipid biosynthesis** and **nitrogen assimilation**.
- Some of the reducing power generated by this pathway may **contribute to cellular energy metabolism**; that is, electrons from NADPH may end up reducing O_2 and generating ATP.
- The pathway produces ribose 5 phosphate, a precursor of the ribose and deoxyribose needed in the **synthesis of RNA and DNA** respectively.
- Another intermediate in this pathway, the four carbon erythrose – 4 – phosphate, combines with PEP in the initial reaction that **produces plant phenolic compounds** including the aromatic amino acids and the precursors of lignin, flavonoids and phytoalexins.
- During the early stages of greening, before leaf tissues become fully photoautotrophic, the oxidative pentose phosphate pathway is thought to be **involved in generating Calvin Cycle intermediates.**

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Lecture-16

INTER RELATIONSHIP OF RESPIRATION AND PHOTOSYNTHESIS

Total dry matter produced by a crop is a function of canopy photosynthesis and canopy respiration. The difference between these two factors gives us is the amount of net photosynthates that are actually available for the growth and development of the plant. Canopy respiration includes both dark respiration and photorespiration.

Survival of any plant is decided by its metabolic activities. Plant needs energy in the form of ATP for these metabolic activities to run. This ATP is generated by the oxidation of the stored food materials like carbohydrates, proteins and lipids. The stored food materials are however produced by photosynthesis. (Fig)

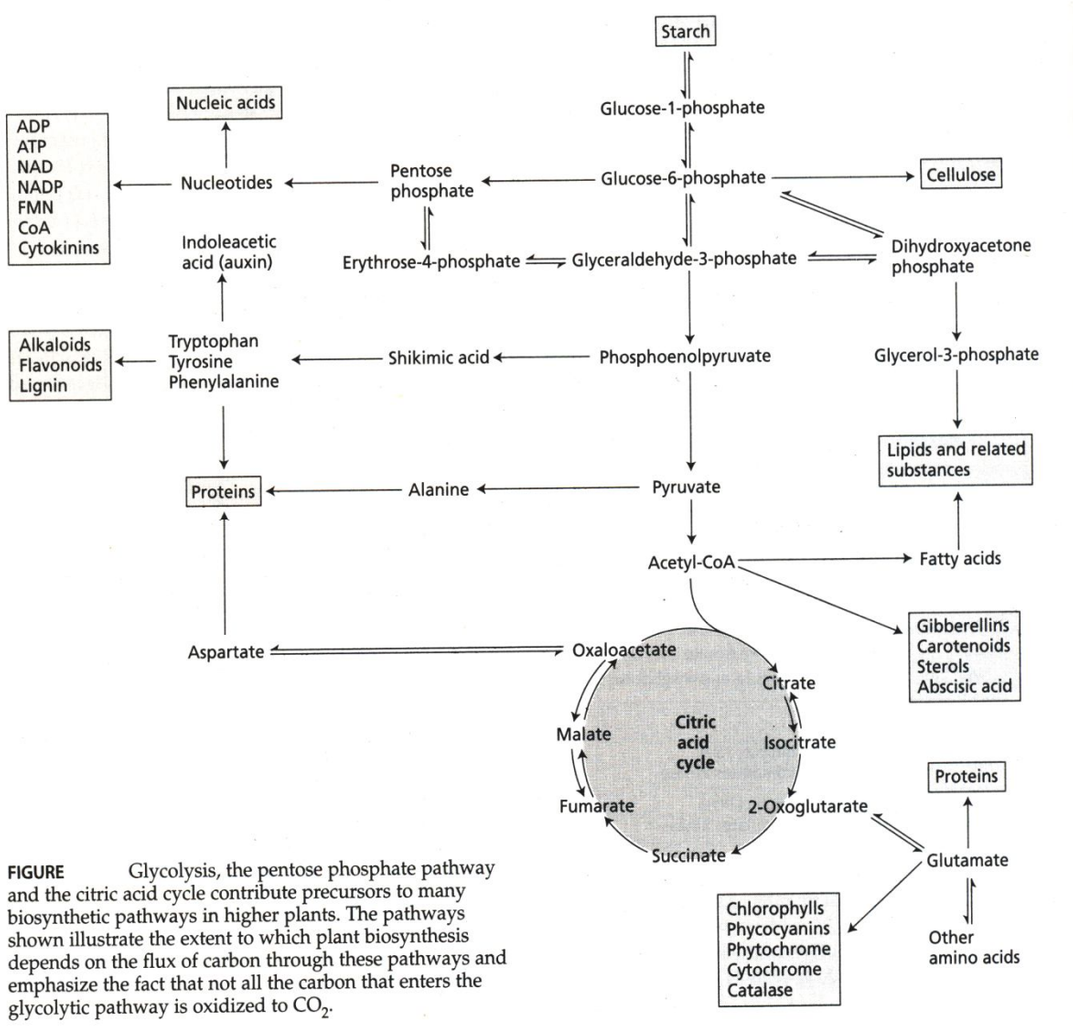


FIGURE Glycolysis, the pentose phosphate pathway and the citric acid cycle contribute precursors to many biosynthetic pathways in higher plants. The pathways shown illustrate the extent to which plant biosynthesis depends on the flux of carbon through these pathways and emphasize the fact that not all the carbon that enters the glycolytic pathway is oxidized to CO_2.

*Figure is from reference 6

Thus, for its survival the plant must always maintain a photosynthetic – respiratory ratio exceeding 1. Otherwise, plants are starved to death. A greater ratio may result from a daily change between relatively cool night temperatures and moderately high day temperatures.

This interrelationship can be better understood from the concept of growth and maintenance respiration.

16.2 GROWTH AND MAINTENANCE OF RESPIRATION:

Respiration provides opportunity for two different sorts of processes in plants: maintenance and growth.

Maintenance is primarily concerned with repair and restoration and the operation of all the metabolic systems necessary for the normal functioning of the plant. These include turnover, transport, maintenance of gradients of all sorts and the requirements for operating controls and signal system. **Growth** is mainly the synthesis and accumulation of all the material and operational systems that constitute the plant.

Growth and maintenance are two quite different processes. American agriculturist **K I MeCree** has shown how they can be quantified independently. Maintenance respiration is clearly a function of plant size (at least in herbaceous plants those do not possess large masses of metabolically inert tissue) so it can be represent as cW. Where “c “is a constant, W is the dry weight of the plant. Growth respiration, however, depends only on the actual new growth being made by the plant. Which is best measured as the net rate of photosynthesis. So growth respiration may be represented of “kP” Where “k” is another constant and P is the rate of Photosynthesis.

Thus the equation for respiration is the **R= kP + cW**.

Therefore, the rate of photosynthesis of a plant should always surpass the total value of growth and maintenance respirations putting together. This is very important for achieving higher yields particularly in root crops and tuber crops where large amount of photosynthates are needed for storage.

16.3 Respiratory Substrates:

A respiratory substrate is any organic plant constituent oxidized partially or completely(to CO_2 and water)in respiratory metabolism. Carbohydrates are the principal respiratory substances in cells of higher plants. The most important respiratory substrates among carbohydrates are Sucrose & starch. In addition of Carbohydrates, other substances also some times serve as respiratory substrates in some plant tissues.

Eg: Castor ⟶ Lipid reserves in endosperm.

CAM plants ⟶ Organic acids (Malic acid, Glycolic acid etc.)

Detached Leaves ⟶ Proteins

16.4 Respiratory quotient: (R.Q)

Respiratory quotient may be defined as the ratio between the volume of carbon dioxide given out and oxygen taken in simultaneously by a given weight of the tissue in a given period of time at standard temperature and pressure.

$$\text{Respiratory quotient} = \frac{\text{Volume of } CO_2 \text{ evolved}}{\text{Volume of } O_2 \text{ absorbed}}$$

For example $C_6H_{12}O_6 + 6O_2 \longrightarrow 6CO_2 + 6H_2O$

In this reaction volume of CO_2 equals to volume of O_2. Thus the RQ of carbohydrates (for example Glucose) is 1.

The following table enumerates R.Q. values of different substrates

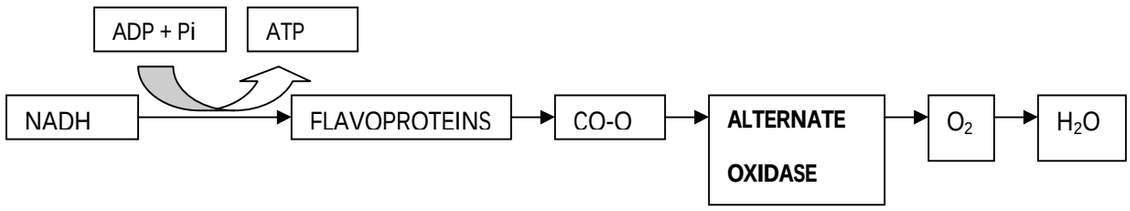
Substrates	**R.Q.**
Carbohydrates	1.00
Proteins when ammonia is produced in oxidation	0.99
Proteins with amide formation	0.80
Fats	0.70
Organic Acids	1.33

16.5 ALTERNATE RESPIRATION/CYANIDE RESISTANT RESPIRATION:

The Alternative Oxidase

If cyanide (1 m/M) is added to actively respiring animal tissues, cytochrome c oxidase is inhibited and the respiration rate quickly drops to less than1 % of its initial level. However, most plant tissues display a level of cyanide- resistant respiration that can represent 10 to 25 %, and in some tissues up to 100 %, of the uninhibited control rate. The enzyme responsible for this oxygen uptake been identified as a cyanide – resistant oxidase component of the plant mitochondrial electron transport chain called the **alternate oxidase.**

It appears probable that mitochondria in tissues exhibiting the alternate pathway have two *parallel* electron transport chains from substrate (e.g., NADH) to O_2 . One is the cyanide – sensitive respiratory chain terminated by cytochrome oxidase. The second (alternate) pathway is terminated by the alternate oxidase. Electrons feed off the main electron transport chain into the alternative pathway at the level of the ubiquinone pool. The alternate oxidase, the only component of the alternative pathway catalyzes a four – electron reduction of oxygen to water.



Alternative pathway contribute to plant metabolism:

One example of the functional usefulness of the alternate oxidase is its activity during floral development in certain members of the Araceae (the arum family) – for example, the voodoo lilly.

Just before pollination tissues of the club like inflorescence, called the *appendix* which bears male and female flowers, exhibit a dramatic increase in the rate of respiration via the alternative pathway. As a result, the temperature of the upper appendix increases by as much as 25^{O} C over the ambient temperature for a period of about 7 hours.

During this extraordinary burst of heat production, certain amines, indoles, and terpenes are volatilized, and the plant therefore gives off a putrid odor that attracts insect pollinators.

It has been suggested that the alternative pathway can function as an "energy over flow" pathway, oxidizing respiratory substrates that accumulate in excess of those needed for the growth, storage, or ATP synthesis.

16.6 <u>SALT RESPIRATION</u>: When roots are absorbing salts, the respiration rate rises. This rise has been linked to the fact that energy is spent in absorbing salts or ions, and that required energy is supplied by increased respiration. This phenomenon called as **salt respiration.**

The inference is that respiration represents the increased metabolism needed to generate energy for the active transport of ions. Unfortunately the relationship is not always linear, and salt respiration may persist after the salts are removed. Consequently, salt respiration does not offer many useful clues to the nature of the coupling of respiration and ion transport.

16.7 <u>WOUND RESPIRATION:</u>

Wounding of a plant organ stimulates respiration in that organ. It initiates meristematic activity in the region of the wound resulting in the development of 'wound callus'. It has been shown that wounding is correlated with an increase in the sugar content of the half cut potato tuber. Perhaps the increase in respiration is due to an increased availability of respiratory substrate in wounded tissues.

16.8 MEASUREMENT OF RESPIRATION:

Methods for measuring the rates of respiration involve quantitative determination of the CO_2 evolved or the oxygen consumed. An individual plant or a plant part or tissue slices, or cell suspension or tissue homogenate or mitochondria forms the sample

DIFFERENT METHODS OF MESUREMENT OF RESPIRATION:

There are five methods of measurement of respiration

A. Weight method: The CO_2 produced during respiration by a plant tissue is trapped in a *barium hydroxide solution.* Weight of the barium carbonate formed indicates the amount of CO_2 produced.

B. Titration Method: The CO_2 produced is made to *dissolve in NaOH* and the amount of CO_2 absorbed is determined by titration.

C. Quantitative method: Release of CO_2 can be measured by passing an air stream through a sealed chamber containing plant tissue into *an alkaline solution* where changes in P^H or E.C. are recorded. From these recorded changes the quantity of CO_2 released by tissue is calculated.

D. Mano metric method: The rates of O_2 uptake or CO_2 release by plant tissue are measured with a mano meter. In this method, sliced samples of plant tissue are suspended in water in a flask. Changes in the amount of respiratory gases with in the flask are reflected in changes in its pressure. These pressure changes can be measured with a manometer.

E. Infra red CO_2 analyzer or paramagnetic O_2 analyzer method: This is a Sophisticated instrument capable of measuring changes in CO_2 or O_2 of the atmosphere and chambers in which plants, or bulky tissues such as large fruits are sealed.

In addition, a membrane bound polarographic electrode sensitive to O_2 can measure changes in the concentration of O_2 in a solution. It should also be mentioned that **mass spectrometric methods** are often used in the recent years to study the gas exchange between plant tissues and the external atmosphere.

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Lecture: 17

NUTRIO PHYSIOLOGY

17.1 DEFINITIONS:

Nutriophysiology deals with metabolic and biochemical functions of the chemical elements and their interaction with other aspects of plant physiology and plant biochemistry. It deals with initial acquisition of chemical elements, their distribution with in the plant and interactions of the plant with its chemical media.

The supply and absorption of chemical compounds needed for growth and metabolism of plants may be defined as **Plant Nutrition** and the chemical compounds required by the plants are termed as **Nutrients**.

Higher plants are autotrophic organisms that can synthesize their organic molecular components out of inorganic nutrients obtained from their surroundings. For many mineral nutrients, this process involves absorption from the soil by roots and incorporation in to the organic compounds that are essential for growth and development. This incorporation of mineral nutrients into organic substances such as pigments, enzyme cofactors, lipids, nucleic acids and amino acids is termed as **nutrient assimilation.** Nutrition and metabolism are thus very closely related.

17.2 ESSENTIAL PLANT NUTRIENTS AND CRITERIA OF ESSENTIALITY:

Plants contain about 80 to 90 percent of water by weight and the remaining 10 to 20 percent is the dry weight. The dry matter consists of a number of organic compounds such as carbohydrates, proteins, lipids and others. Nearly 90 percent of the dry weight of the plant consists of carbon, hydrogen and oxygen. All the mineral elements together contribute only about 6 percent of the dry weight of the plant.

The finding of certain element in plant does not signify that this element is essential for the growth of the plant. For finding out whether an element is essential or not, **Arnon and Stout** (1939) proposed the **criteria of essentially**. These criteria are as follows.

A. A deficiency of the element makes it impossible for a plant to complete its life cycle.
B. The functions of an element cannot be replaced by another element.
   However, recent studies have shown that some of these elements can be partially replaced by others for example magnesium (Mg) by manganese (Mn) and Potassium (K) by rubidium (Rb)
C. The essential element must be directly involved in the metabolism of plant or it may be required for the activation of an enzyme system.

Based on the above criteria the following elements are now known to be essential for higher plants.

Carbon	C	Magnesium	Mg
Hydrogen	H	Iron	Fe
Oxygen	O	Manganese	Mn
Nitrogen	N	Copper	Cu
Phosphorus	P	Zinc	Zn
Sulphur	S	Molybdenum	Mo
Potassium	K	Boron	B
Calcium	Ca	Chlorine	Cl
		Sodium	Na
		Silicon	Si
		Cobalt	Co

17.3 MACRO AND MICRO NUTRIENTS:

Based on the element concentration in plant material, the essential plant nutrients may be divided into **macronutrients** and **micronutrients**. Macronutrients are found or needed in relatively higher amounts than micronutrients. For example, the content of the macronutrient N is thousand times grater than the content of micronutrient Zn in plant tissue. Following this classification C,H,O,N,P,K,Ca,S,Mg,(Na,Si) may be defined as macronutrients. The micronutrients are Fe,Mn,Cu,Zn,Mo,B,Cl.

BENEFICIAL ELEMENTS:

Sodium has beneficial effect and in some cases it is essential. There are some plant species, particularly the chenopodiaceae plants and species adapted to saline conditions that take up this element in relatively high amounts. Na is also required for turnips, sugar beets and celery. The same is true for Si, which is an essential nutrient for rice. Cobalt is an essential element for the growth of the blue-green algae, but it has not been shown to be essential for other algae or for higher plants. It is also required by certain legumes to fix atmospheric nitrogen. Here, however the cobalt ion is necessary for the symbiotic bacteria present in the nodules associated with the roots.

This division of the plant nutrients into macro and micro nutrients is some what arbitrary and in many cases differences between the contents of macronutrients and micronutrients are considerably lower than the example cited above. For example, the Fe or Mn content of plant tissues is some times nearly as high as the content of S or Mg. The content of micronutrients is often far in excess of their

physiological requirements. In many plant species, chloride also occurs comparatively in high concentrations. Yet it is only needed in minute quantities in photosynthesis. This particular example demonstrates clearly that the content of plant nutrient in plant organs (Leaves, stems, fruits, roots) does not give any indication of the quantity effectively needed for physiological and biochemical process.

17.4 CLASSIFICATION OF PLANT NUTRIENTS BASED ON THEIR BIOCHEMICAL ROLE AND PHYSIOLOGICAL FUNCTION: Essential elements are now classified according to their biochemical role and physiological function. Based on the biochemical behavior and physiological functions, plant nutrients may be divided into four groups.

Nutrient elements	Uptake	Biochemical function
1st group C,H,O,N,S	In the form of CO_2, HCO^-_3, H_2O, O_2, NO_3, NH^+_4, $N_2SO_4^{2-}$, SO_2. The ions from the soil solution, the gases from the atmosphere.	Major constituents of the organic compounds of the plant. Essential elements of atomic groups which are involved in enzymatic processes. Assimilation by oxidation reduction reactions.
2nd Group P, B, Si	In the form of phosphates, boric acid or borate, silicate from the soil solution.	They are important in energy storage reactions or in maintaining structural integrity. Elements in this group are often present in plant tissues as phosphate, Borate and silicate esters in which the elemental group is bound to the hydroxyl group of an organic molecule (i.e sugar-phosphates) (Esterification*). The phosphate esters are involved in energy transfer reactions.
3rd Group K, Na, Mg, Ca, Mn, Cl	In the form of cations from the soil solution except chlorine	Present in plant tissues as either free ions or ions bound to substances such as the pectic acids present in the plant cell wall. Of particular importance of their roles as enzyme cofactors and in regulation of osmotic potentials.
4th Group Fe, Cu, Zn, Mo	In the form of ions or chelates from the soil solution	Present predominantly in a chelated form Incorporated in prosthetic groups. Enable electron transport by valency change.

*__Esterification:__

Compounds formed by condensation of an acid and alcohol with elimination of water ADP + Pi = ATP

(**Source** : Taiz and Zeiger 2002)

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Lecture-18

NUTRIOPHYSIOLOGY

18.1 PHYSIOLOGY OF NUTRIENT UPTAKE

Mineral nutrients are found either as soluble fractions of soil solution or as adsorbed ions on the surface of colloidal particles. Various theories proposed to explain the mechanism of mineral salt absorption can be placed in two broad categories:

I) Passive Absorption

II) Active Absorption

ION UPTAKE IS BOTH ACTIVE AND PASSIVE:

After several decades of research on this process of ion uptake it is now believed that the process involves both passive and active uptake mechanisms.

Whether a molecule or ion is transported actively or passively across a membrane (casparian band, plasma membrane or tonoplast) depends on the **concentration** and **charge** of the ion or molecule, which in combination represent the **electrochemical driving force**.

Passive transport across the plasma membrane, occurs along with the electrochemical potential. In this process Ions and molecules diffuse from areas of high to low concentrations. It does not require the plant to expend energy.

Active transport: In contrast, to passive transport energy is required for ions diffusing against the concentration gradient (electro chemical potential). Thus active transport requires the cell to expend energy.

18.1.1 PASSIVE TRANSPORT MECHANISM:

A) Diffusion: Simple **diffusion** to membranes occurs with small, non polar molecules (i.e O_2, CO_2). In this process ions or molecules move from the place of higher concentration to lower concentration. It needs no energy.

B) Facilitated diffusion: For small polar species (i.e H_2O, Ions and amino acids) **specific proteins** in the membrane facilitate the diffusion down the electrochemical gradient. This mechanism is referred to as **facilitated diffusion**.

a) Channel proteins: The specific proteins in the membrane form channels (**channel proteins**), which can open and close, and through which ions or H_2O molecules pass in single file at very rapid rates.

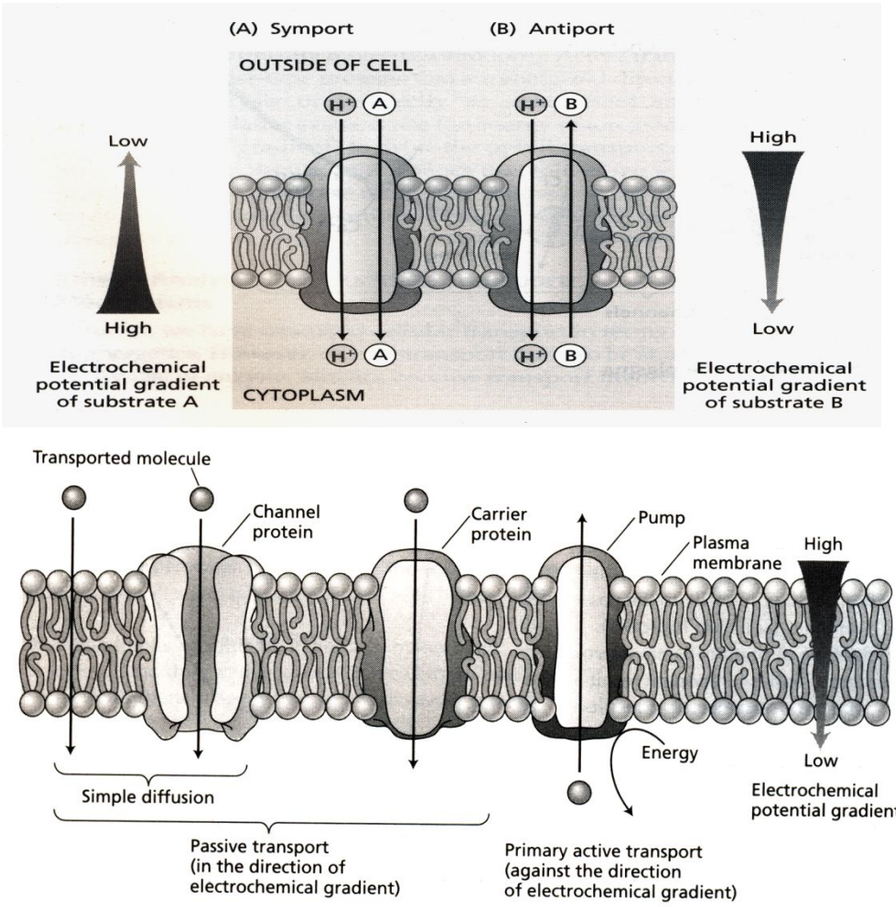
A K^+ and NH_4^+ channel also operates by the same process of facilitated diffusion. In addition Na^+ can also enter the cell by this process.

b) Transporters or Contransporters: Another mechanism involves *transporters or contransporters* responsible for the transport of ions and molecules across membranes. Transporter proteins, in contrast to channel proteins, bind only one or a few substrate molecules at a time. After binding a molecule or ion, the transporter undergoes a **structural change** specific to a specific ion or molecule. As a result the transport rate across a membrane is slower than that associated with channel proteins.

Three types of transporters have been identified.

- **Uniporters** transport one molecule (i.e. glucose, amino acids) at a time down a concentration gradient.
- **Antiproters :** catalyze movement of one type of ion or molecule against its concentration gradient. This is coupled with the movement of a different ion or molecule in the **opposite direction**. Examples of antiporter transport are H^+ - Na^+ and H^+ - Ca^{+2} transport into the vacuole.
- **symporters** catalyze movement of one type of ion or molecule against its concentration gradient coupled to movement of a different ion or molecule down its concentration gradient in the **same direction**. The high H^+ concentration in the apoplast provides the energy for symporter transport of NO_3^- and the other anions.

Therefore, the energy for *antiporter and symporter* transport originates from the **electric potential** and/or **chemical gradient** of a secondary ion or molecule, which is often H^+.



*Figures are from reference 6

18.1.2 ACTIVE TRANSPORT MECHANISM: Larger or more-charged molecules have great difficulty in moving across a membrane, requiring active transport mechanisms (i.e., sugars, amino acids, DNA, ATP, ions, phosphate, proteins, etc.) Active transport across a selectively permeable membrane occurs through **ATP-powered pumps** that transport ions against their concentration gradients. This mechanism utilizes energy released by

hydrolysis of ATP. The Na^+ - K^+ ATP pump transports K^+ into the cell and Na^+ out of the cell, another example is the Ca^{+2} ATP pump.

Thus, it can be understood from the above discussion that the ion transport mechanisms operate both actively and passively. For some of the ions the uptake mechanism is active and for some others it is passive.

18.2 FUNCTIONS OF ESSENTIAL ELEMENTS

The functional roles of essential elements in plants vary greatly from element to element.

A) Nitrogen

Nitrogen is the fourth most abundant element in plants following C, O and H.

1. N is a major structural constituent of the cell. The cytoplasm and the cell organelles contains varying amount of nitrogen largely in combination with C, H, O, P and S.
2. It is an essential constituent of the different types of metabolically active compounds, like amino acids, proteins, nucleic acids, prophyrins, flavins, enzymes and co-enzymes.
3. Being essential for the formation of protoplasm, the deficiency of N inhibits cell enlargement
4. As much as 70 Per cent of the total leaf N may be in chloroplast. Thus the early symptoms of N deficiency are **general yellowing or chlorosis**.
5. Plants contain about 1 to 3 per cent of N on dry weight basis.

B) Phosphorus

1. It is a structural component of the membrane systems of the cell and the mitochondria.
2. It is an essential constituent of nucleoproteins, organic molecules (ATP, ADP etc) which play an important role in the **energy transfer reactions** of cell metabolism, nucleic acids, and coenzymes like NADP.
3. Phosphorus in the phytin of seeds is regarded as a reserve.
4. The unique functions of phosphate in metabolism are its formation of pyrophosphate bonds which allow energy transfer, Uridine triphosphate (UTP) Cytidine triphosphate (CTP) and guanosine triphosphate (GTP) are involved in the synthesis of ribonucleic acids (RNA).
5. Being a constituent of ADP, Phosphoglyceradehyde and ribulose phosphate, P is involved in the basic reactions of photosynthesis.
6. Phosphorus is relatively more abundant in the growing an storage organs.

C) Potassium:

1. K plays a significant role in **stomatal opening and closing**. The mechanism of stomatal closure and opening depends entirely on the K flux.

2. K^+ enhances the translocation of assimilates and promotes rate of Co_2 assimilation.
3. K activates the number of enzymes involved in incorporation of amino acids into proteins and the synthesis of peptide bonds.
4. Potassium regulates the membrane permeability and keeps the protoplasm in proper degree of hydration.
5. Potassium is known to increase the resistance of plants to moisture stress, to heat and to diseases caused by pathogenic fungi and other micro organisms.
6. Inadequate K restricts the formation of xylem and phloem tissue. Lignification of the vascular bundles is generally impaired by K^+ deficiency. This effect probably makes K deficient crops more prone to lodging.

D) Calcium

1. Calcium is required for cell elongation and cell division
2. Calcium plays an essential role in biological membranes. Calcium is deposited in the cell wall as calcium pectate. Ca deficiency obviously impairs membrane permeability and membranes become more leaky.
3. **Germination and growth of pollen** as well as the growth of rhizobium root nodules is affected under low levels of Ca^{2+} supply.
4. Ca appears to play a role in the inhibition of abscission and delays leaf senescence.
5. Calcium is an essential co-factor or an activator of a number of enzymes concerned with hydrolysis like lipase and α-amylase
6. Ca is a structural component of the chromosomes. Where in it possibly binds the DNA to protein. Ca deficiency is known to result in chromosomal abnormality.
7. Calcium plays an important role in neutralizing acids. Particularly citric acid, malic acid, oxalic acid which may become injurious to plants.

E) Magnesium

1. The most well known role of magnesium is **its occurrence at the centre of the chlorophyll molecule**. It is therefore essential for photosynthesis.
2. Mg is a constituent part of the chromosomes and also **essential constituent of poly ribosomes**, the key organelle concerned in protein synthesis.
3. Magnesium is known to play a catalytic role as an activator of a number of enzymes, most of which are concerned in carbohydrate metabolism, phosphate transfer and decarboxylations. The enzyme inorganic pyrophosphatase, as such is inactive. It becomes functional only in the presence of Mg.
4. It is also required for the activation of the enzyme **RuBP carboxylase**.
5. Nitrogen metabolism is also influenced by Mg nutrition.

F)Sulphur

Plants mainly absorb S in the form of SO_4^{2-}

1. Sulphusr is a constituent of amino acids,**cystine, cysteine, and Methionine**.
2. The characteristic odour of cuciferous plants , onion and garlic is due to the presence of sulphur as a constituent of volatile oils.
3. Several other biological active compounds like vitamins (Thiamine and biotin), lipoic acid, acetyl co-enzyme A, ferredoxin and glutathione contain sulphur as an essential part.
4. The active adenosine-5-phospho sulphate (APS) is an important sulphate donor which is involved in the synthesis of glycosides in mustard oil. Being involved in the activation of number of enzymes, participating in the dark reactions of photosynthesis, sulphur is involved in carbohydrate metabolism of the plants.
5. The total S content in plant tissues is in the order of 0.2 to 0.5 percent in the dry matter.

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Lecture:19

NUTRIOPHYSIOLOGY

19.1 FUNCTIONS OF PLANT MICRO NUTRIENTS

A. IRON

1. Iron is a constituent of cytochromes, ferredoxin, catalase and peroxidase. The cytochromes(cyt b_6 and cyt-f) play an important role in electron transport process of oxidative phosphorylation (respiration) and photo phosphorylation (photosynthesis). The iron containing protein ferredoxin plays an important role in the reduction of **CO_2**, atmospheric nitrogen and of sulphate.
2. The leghaemoglobin present in the root nodules of leguminous crops contains iron as an essential constituent.
3. Although iron is not a component of the chlorophyll molecule, it is essential for its synthesis. It has some role in the synthesis of the chlorophyll precursor protoporphyrin –IX. Most of the iron (60-80% of the iron content of the leaves) is found in the chloroplasts.
4. The important iron containing enzymes of higher plants is succinic dehydrogenase, cytochrome oxidase, catalase, peroxidase and aconitase.

B. MANGANESE

1. Manganese is believed to be specific activator of some enzyme like oxidases, peroxidases, dehydrogenases and kinases.
2. Manganese is known to be a constituent of nitrite reductase and hydroxylamine reductase, both of which are concerned in nitrogen assimilation. In the absence of manganese, nitrite accumulates and leaves show symptoms of nitrogen deficiency. Manganese regulates the reduction of nitrite into hydroxylamine and subsequently into ammonia by influencing hydroxylamine reductase.
3. Manganese plays a key role in carbohydrate metabolism and also affects the absorption of calcium and potassium ions.
4. Manganese is involved in the oxygen evolving step in photosynthesis –of **PSII (water oxidizing enzyme complex)**

C. COPPER

1. The copper containing compounds plastoquinones and plastocyanins are involved in the electron transport from chlorophyll to NADP during the primary reactions in photosynthesis. Thus copper stress in plants has been shown to result in a decreased rate of photosynthesis.
2. Copper is a constituent part of several enzymes like nitrite reductase, cytochrome oxidase and ascorbic oxidase.

3. Relatively, high concentrations of Cu occur in chlorophasts. About 70 percent of the total Cu in the leaf is found in these organelles.

D) <u>BORON</u>

1. Boron is associated with the reproductive phase in plants and its deficiency is often found to associate with **sterility and malformation** of reproductive organs. Boron is thus required for the germination of pollen and growth of pollen tube.
2. Boron plays an important role in carbohydrate metabolism, particularly in the **translocation of photosynthates**. Boron was considered to be involved in the translocation of sugars in the form of **sugar borate complexes.** These complexes pass more rapidly through cell membranes than the free sugars.
3. Boron is required for the proper development and differentiation of vascular elements.

E) <u>ZINC</u>

1. Zinc is a metal component of a number of metallo enzymes like **alcohol dehygenase** and **lactic dehydrogenase**.
2. Zinc is essential for the synthesis of the amino acid **tryptophan**, a precursor of an important plant growth hormone indole acetic acid (IAA).
3. Zinc is closely involved in N-metabolism of the plant.
4. Zinc plays a role in plant metabolism involved in starch formation. **Zinc** along with **Cu** has been shown to be a constituent of the enzyme **super oxide dismutase**.

F) <u>MOLYBDENUM</u>

1. Mo is an essential component of two major enzymes in plants *viz.,*nitrate reductase and nitrogenase. Nitrate reductase concerned with the reduction of nitrate to nitrite in both microorganism and higher plants.

   Nitrogenase consists of two enzyme protein complexes, the bigger of which contains Fe and Mo in a ration of about 9:1
2. By virtue of being a constituent of nitrate reductase, Mo plays direct role in nitrogen metabolism of plants.
3. Mo is known to be a specific inhibitor of acid phosphatase.

G) <u>CHLORINE</u>

1. Chlorine has been shown to be involved in the oxygen evolution in photo system II in photosynthesis (Cl and Mn are important for this reaction)
2. It raises the cell osmotic pressure.
3. Chlorine accelerates the activation of amylase which converts starch into soluble sugars.

19.2 FUNCTIONS OF BENEFICIAL NUTRIENTS

a) COBALT

1. Cobalt is required by rhizobia for the fixation of elemental nitrogen both by leguminous and non-leguminous symbionts.
2. It is a structural component of **vitamin B_{12} (cyanocobalamine).**
3. B_{12} is essential for the formation of hemoglobin concerned in nitrogen fixation.

b) SODIUM

1. In higher plants, sodium has so far been shown to be essential only for two halophytic species *Atriplex vasicaria* and *Hologeton glomeratus*.
2. The best known role of sodium is in the maintenance of osmotic relations of the cell.
3. Sodium has beneficial effect on growth and water relations of sugar beet.

c) SILICON

1. The effect of Si is especially important in the yield and quantity of the **rice** crop.
2. Recent studies have shown that, Silicon imparts disease resistance and lodging resistance in paddy
3. The grain yield of the plants with Si is twice more than the plants without Si.
4. The concentration of Si in rice will be around 100 mg g^{-1}.

19.3 MOBILITY (PHLOEM TRANSPORT) OF INORGANIC SOLUTES

The mineral nutrients initially acquired by the roots move up ward in xylem. Many of them are then subjected to redistribution via the phloem but a few are not. Immobility in the phloem presumably is caused by failure of these elements to enter the sieve tube.

Bukovac and Wittwer (1957) studied the mobility of many radio actively labeled mineral elements applied to leaves of bean plants and classified them into three groups based on the mobility in phloem.

Mobile	**Intermediate**	**Immobile**
Nitrogen	Iron	Calcium
Phosphorus	Manganese	Boron
Potassium	Zinc	
Magnesium	Copper	
Chlorine	Molybdenum	
Rubidium		
Sodium		
Sulphur		

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Lecture-20

NUTRIOPHYSIOLOGY

20.1 DEFICIENCY SYMPTOMS OF PLANT NUTRIENTS

A) NITROGEN

Nitrogen being a mobile element within a plant, it's deficiency results in movement of N from older to younger (upper) leaves. As a result the older leaves turn yellow in colour.

1. In young plants, growth is stunted with yellowish green leaves. Yellowing of leaves is due to the collapse of chloroplasts resulting in decrease of chlorophyll content.
2. Yellowing always starts from the older leaves and spreads to young ones.
3. Severe N deficiency affects the leaf tissue to become dry and necrotic.
4. Older leaves are shed prematurely.
5. Root growth is affected and branching is restricted.
6. Shoot become short, thin with up right growth and spindly appearance.
7. Flowering is reduced.
8. In cereals, tillering is poor, number of ears per unit area and number of grains per ear head is reduced.

B) PHOSPHORUS

Generally the symptoms of P deficiency appear in the older leaves.

1. Young plants are stunted with **dark blue green leaves**.
2. Stems become slender
3. Root system is limited. Primary and secondary roots elongate in length with short tertiary roots.
4. In many plant species P deficiency induces the **formation of anthocyanin pigment** and the leaves acquire **purple color** primarily along margins and on the lower stalk (eg. Maize)
5. The formation of fruits and seeds is depressed and ripen slowly.
6. Plant often dwarfed at maturity.
7. In potato, tubers may develop rusty lesions in the flesh.

C) POTASSIUM

1. The most characteristic symptom of K deficiency is **tip and marginal scorching** of most **recently matured leaves**.
2. In **barley** which is the most susceptible of the cereals, numerous small brown areas develop in the areas between the veins.
3. Roots are slender and poorly developed in sugar beet.
4. In certain temperate fruit trees, K deficiency may result in severe "die-back"
5. Resistance of plants to infection by bacterial and fungal pathogens is reduced.

6. Putriscine, (a Diamine) accumulates under K^+ deficiency.

D) CALCIUM

1. Symptoms of Ca deficiency appear earliest and severely in **meristametic regions and young leaves** because it is **immobile** one.
2. Breakdown of meristemetic tissues in stems and roots occur.
3. Roots poorly developed, lack fiber and may appear gelatinous.
4. The apical bud "**dies off**" and thus small branches arise from lower leaf axils giving the plant bushy appearance.
5. Little or no fruiting.
6. Calcium deficiency causes **"bitter pit"** disease in apple and **"blossom end rot"** in tomato and watermelon.

E) MAGNESIUM

1. **Interveinal yellowing or chlorosis** occurs in **older leaves** and under severe deficiency the areas become necrotic.
2. In **cotton**, **Mg deficiency** induces formation of **anthocyanin pigments** and a **reddish coloration** of leaves during winter.
3. In **citrus**, the chlorosis commences at the tips and margins of the leaf and spread in ward towards midrib by leaving **inverted V shape** at the bottom of the leaf.
4. In general fruit trees are susceptible to Mg deficiency and show varied patterns of chlorosis, necrosis and pigmentation of old leaves which shed pre maturely.

F) SULPHUR

1. Chlorotic symptoms first appear in younger most recently formed leaves.
2. Shoot growth is more affected than root growth.
3. Leaves become narrow and chlorotic in cruciferae.
4. Stems often become slender.

G) IRON

1. Being relatively immobile, Fe deficiency appears first on the younger leaves of the plant.
2. In most species chlorosis is interveinal and a fine reticulate pattern can be observed in the newly formed leaves, the darker green veins contrasting markedly against a lighter green or yellow background. The youngest leaves may often be completely white and totally devoid of chlorophyll.
3. In the leaves of cereals the deficiency is shown by alternate yellow and green strips along the length of the leaf.

4. In fruit trees, iron deficiency is associated with high leaves of Ca Co_3 in the soil, where it is called **"lime induced iron chlorosis"**

H) MANGANESE

1. Mottled chlorosis with veins green which appears first on younger leaves.
2. Stem becomes yellowish green, often hard and woody.
3. In **groundnut** leaves, the **veins are surrounded by a definite zone of non chlorotic interveinal tissue**.
4. Characteristic symptoms of Manganese deficiency in certain crops have been given specific names. They are **grey speck of oats**, **speckled yellow of sugar beet**, **marshy spot of peas**, **pahala blight of sugarcane etc.**

I) BORON

1. B is **immobile** in most plant species and symptoms appear first at tops and roots.
2. The youngest leaves are misshaped, wrinkled and are thicker and of a **dark bluish green color**. Shed prematurely.
3. Plants dwarfed, stunted, flower development and seed production usually impaired.
4. In fruit trees, the bark of the stem may become rough and show splitting. Fruits often develop corky areas internally.
5. Roots exhibits swollen root tips.
6. Characteristic symptom of B deficiency in certain crops are

Heart rot in sugar beet	**Hollow stem in cauliflower**
Brown heart of turnip	**Corky pith of apples**
Hard fruit of citrus	

J) COPPER

1. Young leaves show chlorosis
2. Terminal leaves, shoots are wilted and distorted, frequently followed by death resulting in development of several auxillary buds.
3. Cereals show bushy growth with white twisted tips and reduced panicle formation.
4. Poor or no heading in cabbage and lettuce.
5. In citrus, Cu deficiency leads to **exanthema or die-back.**

K) ZINC

1. **Little leaf and rosette** are the common symptoms of Zn deficiency.
2. In maize, Zn deficiency results in white or yellow emerging leaves, a condition called **white bud**.

3. Leaves chlorotic and necrotic, young growth first affected, resulting, premature shedding.
4. In Rice Zn deficiency causes ‘khaira disease’
5. In monocots and particularly in maize, **chlorotic bands form on either side of the midrib of the leaf**.

L) MOLYBDENUM

1. Symptoms of Mo deficiency are similar to those of N deficiency because in the absence of Mo, nitrate is not metabolized.
2. Leaves show marginal scorching and rolling or cupping.
3. In extreme deficiency, the leaf lamina is not formed and only the rib is present in **cauliflower** which is called **whiptail disorder**.

M) CHLORINE

1. Chlorosis of younger leaves and an overall wilting of the plant.
2. In some plant species, like tomato, leaves show chlorotic mottling, bronzing and tissue necrosis.

N) SILICON

1. Withering of leaves and wilting of plants, which are called **weeping willow habit of growth**.
2. In cereals and grasses, necrotic spots develop on leaves.

20.2 NUTRIENT DEFICIENCIES AND SOME SPECIFIC NAMES

Specific names have been given to symptoms of certain nutrient deficiencies. They are:

Calcium	Bitter pit in apple, Blossom end rot in tomato
Manganese	Grey speck of oats, speckled yellow of sugar beat, Marsh spot of pea pahala blight of sugarcane, Frenching of tung tree.
Boron	Heat rot in sugar beat, Browning or hollow stem in cauliflower, Top sickness of tobacco, Brown heart of turnip, Cracked stem of celery, Corky pith of apple, hard fruit of citrus.
Copper	Exanthema or dieback in citrus
Zinc	White bud in maize, khaira disease in Rice.
Molybdenum	Whiptail in cauliflower

20.3 TOXICITY SYMPTOMS OF PLANT NUTRIENTS

As excess supply of one or more of the essential elements, particularly the heavy metals brings about a reduction in growth and often leads to the production of visual symptoms which at times are characteristic of the elements supplied in excess. The visual effects of excess supply of elements may be due to a direct effect of toxicity of elements or by inducing deficiency of another element. The excess supply of particulars elements reduces the uptake and utilization of other essential elements. Eg. Excess phosphorus supply may retard the uptake and translocation of Cu and Zn, thus shows Cu and Zn deficiency symptoms.

Under field conditions N, S and Al toxicities are common. Mn toxicity is observed in acid soils.

A) NITROGEN

1. As a Nutrient – excessive nitrogen commonly produces plants that vegetative so that yield is reduced. In sugarcane both juice quality and sugar recovery is reduced. Fruit quality is impaired in organs and peach. Cereals receiving excess N have been shown to exhibit lodging as a result of the decreased thickness of the cell walls or due to the poor development of the mechanical tissues of the stem or due to both. In tomato, plants are highly vegetative and fruit production is reduced.
2. As a salt a high level of N application increases the salinity of the soil. Also the form of nitrogen (NH_4^+ or NO_3^{--}) and associated ions (SO_4^-, NA^+ Ca^{++}) may markedly affect soil structure and plant response.

B) SULFUR

Excess sulfur causes interveinal yellowing in sorghum and lemon leaves. No symptoms of S toxicity is observed in leaves of sugar beet, tomato, cotton and alfalfa, but growth and leaf size was considerably reduced in all the crops

Excessive concentrations of sulfur dioxide in the atmosphere also cause toxicity in the plants. The symptoms produced by excessive So_2 was divided into (a) acute injury (b) Chlorotic injury.

The acute injury consists of collapsed marginal or interveinal areas which at first have a dull, water soaked appearance, later drying and bleaching to an ivory color in most species. These lesions are caused by rather sudden absorption of enough gas to kill the tissue.

Chlorotic injury is a yellowing of the leaf which may progress slowly and bleaches completely. bleaching most of the chlorophyll and carotenoids . This is due to absorption of an amount of gas somewhat insufficient to cause acute injury or it may be caused by absorption over a long period of time of sublethal amounts of gas.

Another type of S toxicity is Hydrogen sulfide toxicity (sulfide injury). In **poorly drained rice soils, the H_2S toxicity** is common. This disorder is commonly called as **“akagare”** or **“akiochi”.** This anaerobic disorder is characterized by inhibited root development and browning (or blackened) and death of roots which

precede the stunting of shoots. Field observation shows that the roots of affected plants are blackened from the accumulation of iron sulfides (and possibly manganese sulfides) formed under reducing conditions. This black coloring disappears upon exposure to the atmosphere after several hours due to oxidation, a confirmation of sulfide formation. The H_2S toxicity also depresses the uptake and translocation of P and other nutrients.

C) <u>ALUMINUM</u>

Aluminum toxicity is wide spread in most of the acidic soils. In general, roots are affected first, more severely than the tops under aluminum excess. In most species, roots appear thick and brown and the tips of the root lets often appear enlarged. In barely, the general growth of the plants and tillering are severely restricted. Leaf tips often turn brown and wither. The stem becomes dark brown, no ears are formed and plants often die prematurely. The root growth is severely restricted and the root tips appear dark brown and enlarged.

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Lecture-21

NUTRIOPHYSIOLOGY

21.1 FOLIAR NUTRITION

Foliar nutrition of mineral nutrients by means of sprays offers methods of supplying nutrients to higher plants more rapidly than methods involving root application. Foliar nutrition consists of spraying dilute nutrient solutions on foliage in order to feed the plants with required nutrients in short time.

21.1.1 Mechanism of uptake: In terrestrial plants the uptake of solutes by the surfaces of leaves and other aerial parts is severely restricted by the external wall of the epidermal cells. This wall is covered by a layer of wax and cutin and contains pectin, hemi cellulose and cellulose. The direct penetration of solutes from the leaf surface through open stomata into the leaf tissue is unlikely because a cuticular layer also covers the surface of guard cells in stomatal cavities. Further more ion uptake rates from foliar sprays are usually higher at night, when the stomata are closed, than during the days, when the stomata are open.

The movement of solutes across the cuticular layer takes place in cavities or channels called **ectodesmata** by process of diffusion. Ectodesmata extend through the outer epidermal cell walls from the inner surface of the cuticle to the plasma membrane. When a substance reaches the plasma membrane of an epidermal cell it will be absorbed by mechanisms similar to those which operate in root cells.

21.1.2 Advantage of foliar nutrition: The advantage of foliar nutrition is high recovery rate while the limitation is the smaller amounts of nutrients that can be supplied at a given time. It is very essential not to excess the specified concentration of fertilizer solutions, for the foliar spray to avoid the risk of leaf scorch. Fertilizers when used in excess concentration damage the leaf tissue causing necrosis.

The technique has great practical utility under certain conditions.

a) **Correction of visual symptoms**: Nutrient deficiencies observed during early stages of crop growth can be corrected by foliar sprays.
b) **Low nutrient availability in soils**: in calcareous soils, for example, iron availability is very low and iron deficiency (Lime induced iron chlorosis) is widespread. Foliar spray is much more efficient than the soil application of expensive iron chelates.
c) **Decrease in root activity during reproductive stage**: As a result of sink competition for carbohydrates, root activity and thus nutrient uptake by the roots decline with the onset of the reproductive stage. Foliar sprays containing nutrients can compensate for this decline.

Usually micronutrients, which are required in low rates, are foliar sprayed. Urea sprays are used for supplying nitrogen. Ammonium phosphate fertilizer is also used to spray to improve seed setting in multicut fodder legumes like Berseem.

21.2 HYDROPONICS

The method of growing plants in aqueous nutrient solutions is known as hydroponics culture or hydroponics. The growing of higher plants with their roots in dilute solutions of mineral salts instead of in soils has led to a vastly increased understanding of plant nutrition. The use of water culture technique for growing plants permits precise control of the supply of nutrient ions in the root environment. The first recorded use of water culture technique was in the year 1699 by **John Woodward**. Latter the process of growing plants in water culute was named as hydroponics by **Gericke** 1930.

The water culture technique is extensively used to grow certain high value crops (eg.Tomato, lettuce and strawberry) in glass houses during off seasons in metropolitan areas of developed countries. In addition, vegetables are grown in plastic houses in a few coastal desert regions, where sea water is desalinated and supplied to roots in precisely the amounts required by the growing plants.

21.2.1 TYPES OF SOLUTIONS CULTURE:

The three types of solution culture are

1. Static 2. Flowing 3. Mist system

In static system, the plant is supported in such a way that its roots are immersed in culture solutions containing nutrient elements. The solution is kept oxygenated by bubbling air through it from a compressed air line. This system is mostly used for experimental purpose. In flowing systems, the solution move continuously allowing its composition to remain relatively constant at the root surface. The nutrient film technique, where roots grow in films of solution constantly moving down inclined troughs is a commercial version of the flowing system.

In mist system, roots hang in the air and are sprayed intermittently with a nutrient solution.

21.2.2 SAND CULTURE:

In a modification of the solution culture technique, plants are grown with their roots anchored in a solid inert aggregate, such as sand culture technique. The added advantage of this solid medium culture is that the roots grow in a natural medium and no other means of support needs to be provided.

Most important form of solid culture techniques is sand culture. Sand culture is sometimes referred to as slop culture, drip culture, or intermittent renewal. Plant nutrients are supplied with solutions, as in water culture. The major difference is that the plants are grown in silica sand or other inert material (perlite, vermiculite, peat etc.). The solution is applied to the sand and then allowed to drain off. In this way plant nutrients are supplied, aeration is provided, and the roots of the plant are supported. Additional support may be required for the aerial portions of the plant,

especially tomatoes and cucumbers. Sand should be medium to coarse in size (.0.25 mm-2mm).

Perhaps the most limiting aspect of solid media is that access to roots is achieved only with major damage.

21.2.2.1 Types of solid medium culture

Based on the addition of nutrient solutions to the solid culture the following three systems are categorized.

1. **Slop culture**:- Nutrient solutions are poured over the surface of the solid medium.
2. **Drip culture**:-Nutrient solutions are dripped on the surface.
3. **Sub irrigation**:- Nutrient solutions are forced from the bottom of the container to come up to the surface of the solid medium.

Methodology

Stock solutions are prepared separately from inorganic salts containing the essential elements for normal plant growth. **Hoagland and Arnon** (1950) formulated two nutrient solutions which have been very widely used and the term "**Hoagland solution**" has become a household word in laboratories devoted to plant nutrition all over the world. Hoagland solution II contains ammonium ions as well as nitrate and as a result it is a better buffered one. pH of the nutrient solutions will be in the range of 5 to 7.The final nutrient solution is prepared by adding the correct proportion of stalk solutions and diluted with deionised water. The containers are filled with nutrient solution and covered with aluminum foil or thick non transparent paper in order to eliminate light incidence and algal growth in containers.

Week or ten days old seedling are taken, the roots are submerged in the nutrient solution and the stem projected through an opening cut in the container lid. To ensure the stem to be held rigid, the opening is stuffed with some inert padding material such as cotton. The nutrient solution is well aerated periodically by employing an air-pump and change once in few days depending on undesirable pH changes and the growth rate of the plants.

For commercial cultivation, large shallow tanks (nutrient film technique) are used. The nutrient solutions are replenished often enough to maintain their concentrations. Nutrient solutions are made to flow over the plant roots from a recirculating tank .

Use in Agriculture

1. To grow plants in the laboratory for experimental purpose such as studies involving nutrio physiology, herbicide physiology, radio tracer technique for ion uptake and movement etc.
2. Commercial cultivation of high value crops in glass houses during off seasons in metropolitan areas.

3. Deficiency and toxic symptoms of mineral elements can be developed and studied with different crop plants.

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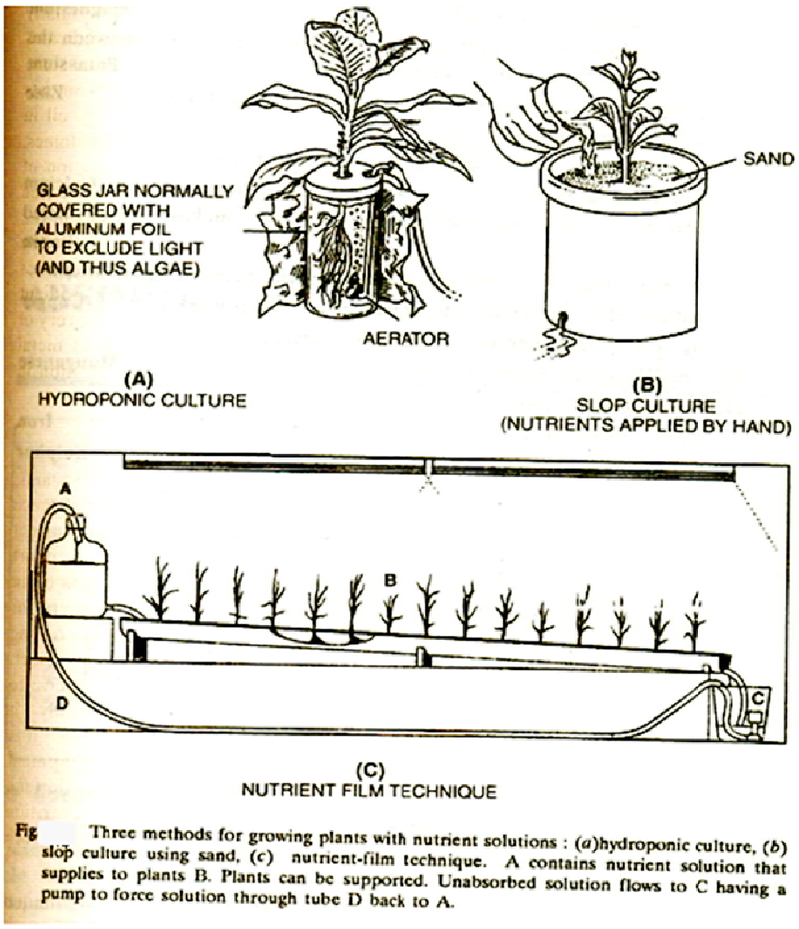

Fig Three methods for growing plants with nutrient solutions : (*a*)hydroponic culture, (*b*) slop culture using sand, (*c*) nutrient-film technique. A contains nutrient solution that supplies to plants B. Plants can be supported. Unabsorbed solution flows to C having a pump to force solution through tube D back to A.

Lecture-22

PHOTOPERIODISM AND FLOWERING

Light controls many physiological processes in the plant among them the process of flowering is important even in the life cycle of the plant. In case of annuals, floral induction signals the end of vegetative growth. In perennials, floral initiation is not accompanied by such changes and the vegetative and reproduction growth occur simultaneously. Reproductive growth is a complex process which includes anatomical, morphological, physiological and biochemical process. All these changes are finally manifested into transformation of vegetative to reproductive bud.

Before floral primordial can be initiated, the plant must complete a period of vegetative growth of attain some minimal leaf number. When this condition is attained the plant is said to be **ripe-to-flower**. In most plants, ripeness to-flower is attained after the plant has produced several leaves. In some annual grasses a minimum of 7 **leaves** must be developed before the plant is ripe to flower. On the other hand, a plant such as ***pharabtis nil*** is ripe to flower within a day after the cotyledons have emerged. The cotyledons presumably contain enough stored food to support subsequent reproductive development.

However, most of the commonly cultivated plants and weedy annuals attain the ripe-to-flower condition **2-3 weeks** after seedling emergence when a few leaves have fully developed.

22.1IMPORTANCE OF PHOTOPERIODISM IN AGRICULTURE:

The knowledge of photoperiodism and its application has great economical potential as follows

- The yield of tubers, corns, bulbs and rhizomes can be increased substantially by increasing or decreasing the duration of day or night.
- Annuals may be grown twice or thrice in a year.
- Perennials might flower throughout the year.
- Hybridization experiments have got a fillip because different varieties growing in different areas with different durations and flowering at different times are made to grow and flower side by side by artificially controlling their photoperiods.
- Understanding the concept of photoperiodism has helped to choose photo insensitive varieties and cultivars. These cultivars are best suited in intensive agriculture.

22.2 CLASSIFICATION OF PLANTS BASED ON PHOTOPERIODIC RESPONSES

Garner and Allard (1920) observed that flowering in Maryland mammoth variety of tobacco (*Nicotiana tabaacum*) and Biloxi soybean (*Glycine max*) can be controlled by artificially increasing or decreasing the natural duration of sunlight. They introduced the term photoperiodsm and defined that as the **response of plant to the relative length of day and night**. However, the duration of dark period is also much important to understand the concept.

Garner and Allard classified the plants into 5 types based as their photoperiod response of flowering

A. Short day plants (SDP) B. Long day plant (LDP) C. Day neutral plant (DNP)

D. Short –long- day plants (SLDP) E. Long- short- day plants (LSDP)

A)Short day plants (SDP)

Plants that readily flower when the day length is less than its critical day length (photoperiod required to induce flowering is critical day length). For example, critical day length for xanthium (SDP) is about 15.5 h and it flowers only when day length is 15.5 h or less It was also shown that even a brief exposure to light during dark period inhibits flowering and therefore it requires a continuous and uninterrupted dark period for its flowering. On the other hand, a brief period of darkness during day time had no effect on flowering. Because of the importance of dark period in flowering, these are also called as **Long Night Plants.**

B)Long day plants (LDP)

These plants flowers readily only when the photoperiods are longer than the critical photoperiod. For example annual *Hyoscyamus* has a critical photoperiod less than 10h.30m. As a result, this plant does not flower with a photoperiod less than 10h.30m but it promptly flower under any length of photoperiod above this limit and even within 24h of light. These plants are also called as **short night plants**.

C)Indeterminate or Day Neutral Plants

Indeterminate or DNP flowers readily over a wide range of day length from relatively SDL to continuous illumination.

D) SLDP

These plants require first short photoperiod and then long photoperiod for flowering.

E) LSDP

These plants require first long days and then short photoperiod for flowering

These plants do not flower when exposed to either SD or LD and need both LD and SD for its flowering

22.2.1 EXAMPLES OF PLANTS WITH DIFFERENT PHOTOPERIOD REQUIREMENT ARE GIVEN BELOW

1. Short day plants

A. Qualitative SDP (Plants specifically requiring SD)

1. Chrysanthemum 2. Tobacco 3.Xanthium 4. Rice *(Oryza sativa)*

5.Japanese morning glory (*Pharbatis nil*)

B. Quantitative SDP (Plants promoted by SD)

1. Cosmos 2. Cotton 3. Hemp

2. Long day Plants

A. Qualitative LDP (plant specifically requiring LD)

1.Barley (winter barley) 2.Wheat 3.Henbane *(Hyoscyamus Niger)*

4.Spinach 5.Sugar beet

B. Quantitative LDP (plants promoted by LD)

1.Lettuce 2.Blue grass 3.Clover

3. Day neutral plants

1.Cucumber 2.Balsam 3.maize 4.Tomato

4. LDSDP

1.Bryophllum 2.*Cestrum Nocturnum* (Night blooming Jasmine)

5. SDLDP

1.Winter rye 2. Candy tuft

22.3 PERCEPTION OF PHOTOPERIODIC STIMULUS

Knott (1934) first observe that photoperiodic induction is perceived by young expanded leaves. Later Knott's work was confirmed by Chailakhyan (1936) he divided chrysanthemum plants into four groups and varied the regime of light

and dark cycles in all the four groups by using light proof cases. The four groups of plants are as follows:

Group A Entire plant continuously received long day treatment

Group B Lower leaf portion received short day treatment while upper defoliated portion received long day treatment

Group C Lower leafy portion received long day treatment while upper defoliated portion received short day treatment

Group D Entire plant continuously received short day treatment

He observed that flowering occurred in those plants where the leaves received short day treatment (Group B&D) but it failed in those plants where the leaves received long day treatment (Group A&C). On the basis of above observation he concluded that short day stimulus is perceived by the leaves.

Floral stimulus

The flowering stimulus produced in the photo induced leaves is translocated to the shoot apices for flower evocation. It has been observed that floral stimulus is similar in long and short day plants by grafting experiments. If a leaf from a photo induced plant is removed and then grafted on a non induced plant, then this plant flowers. Apparently a chemical substance is produced during photo inductive cycle, which is transmitted during grafting to non induced plants and evokes flowering. **Chailakhyan** (1936) named this flower inducing chemical substance as Florigen. The flowering stimulus travels through phloem but independent to the transport of photosynthates.

22.4 Biological clocks:

Study of Biological clocks is also known as chronobiology. The best understood biological clock is the circadian rhythm.

The various physiological processes do not occur at a constant rate during the different times of the day and night. The rate fluctuates showing peaks and dips at regular intervals. In other words the occurrence of the process is rhythmic. At first glance it may appear that such rhythms may be because of different external factors like temperature, light and humidity which fluctuate during the different times of the 24 hrs day. However, the plants continue to exhibit such rhythms even when held at constant conditions. It means that the occurrence of such rhythms is not because of variations in the external conditions (not exogenous) but appears to be controlled by the plant itself (endogenous). They are called as **endogenous rhythms**.

Most of the endogenous rhythms that occur in plants have a periodicity of frequency of about 24 hrs. That is, this process is repeated once in about 24 hrs and called **circadian rhythms**. Some properties of these diurnal rhythms are demonstrated by the sleep movement of the primary leaves of bean (*Phaseolus multiflorus*).(Fig.)

During the day the leaves are horizontal, while during the night the leaves assume a more vertical position. The plant is exposed to normal light and dark period for 2 days and was subsequently kept under light. The primary leaves continue to open and close once in about 24 hrs even continuous light.

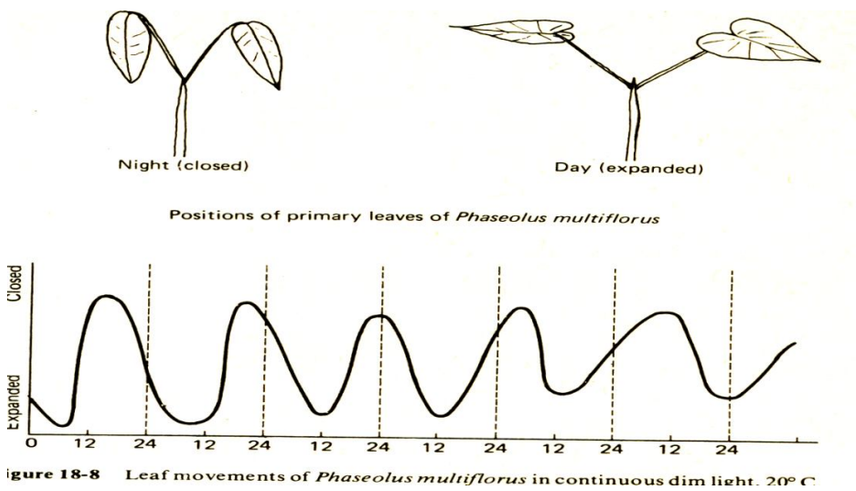

*Figure is from reference.7

The other examples of circadian movement in higher plants are cell mitosis, respiration, CO2 fixation in Bryophyllum, enzymes activity, nectar odor and flower petal movement.

Experiments conducted by De Mairan, Pfeffer, Bunning and Stern and others have clearly shown the existence of an internal (endogenous) time measurement system in plants. The biological clock, as it is called, is believed to be very accurate as it measures the rhythms of a small period as one minute to as large a period as one year. Though the nature of the biological clock or the endogenous factor controlling it is not known, it is suggested to be located within the cell apparatus of even unicellular organisms.

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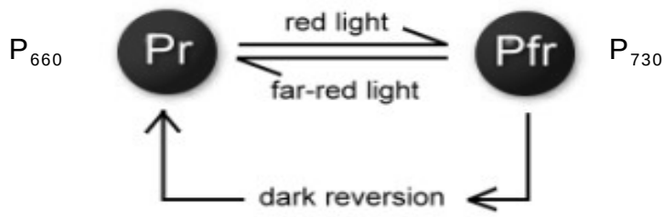
Lecture-23

PHOTOPERIODISM AND FLOWERING

23.1 PHYTOCHOROME

Phyrochrome is a blue proteinaceous pigment composed of two components: a protein and a chromophore - which give its light absorbing properties. **Borthwick and Hendricks** (1946) suggested that phytochrome exists in two forms: a red light absorbing form (pr), blue green in color and a far red light absorbing from (pfr), light green in color. These two forms are inter convertible and it is located within plasma membrane. Phytochrome is produced in light grown tissues:



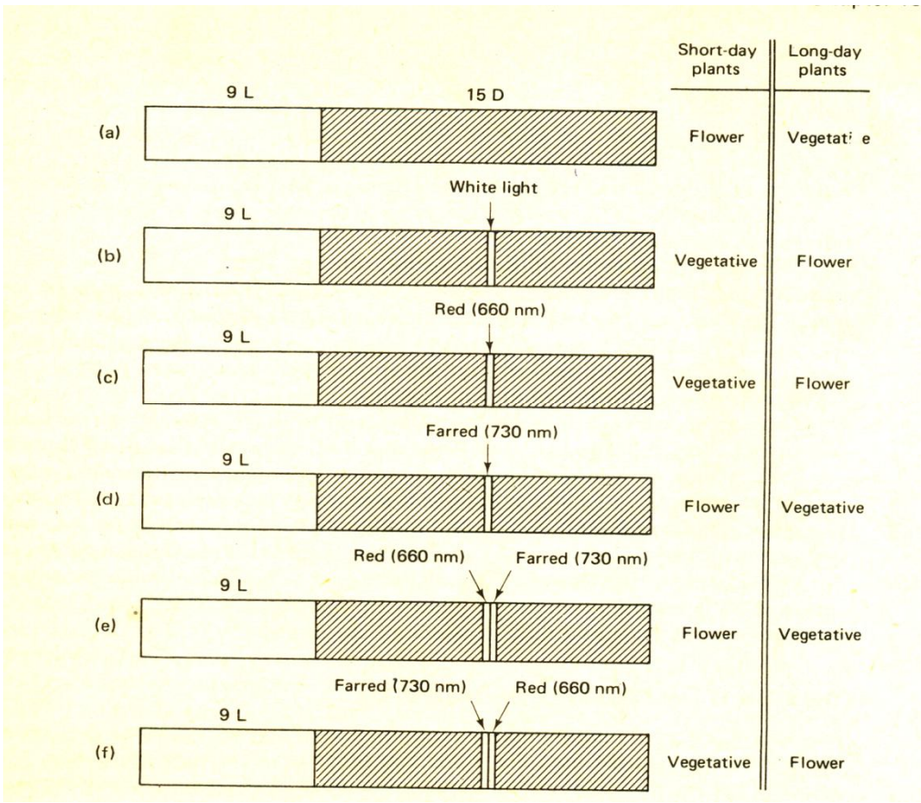
It shows on absorbing red light, P_r form is converted to p_{fr} during day time, while p_{fr} form gradually converted into p_r form in dark on absorbing far red light.

23.1.1 Role of phytochrome in short day plants

At the end of light period the phytochrome is predominantly present in the pfr form and the ratio of p_{fr} to pr is such that, the formation of the flowering stimulus is prevented. During the long dark period, spontaneous conversion of pfr to pr takes place or p_{fr} is destroyed, and the ratio of p_{fr} to pr finally drops to a level where metabolic processes are triggered which leads to the formation of flowering stimulus. This duration represents the critical dark period requirement for the flowering of SDP. It varies from plant to plant. If the dark period is interrupted by flash of red light, pr is converted to p_{fr}. And the p_{fr} to pr level is such that the formation of the flower stimulus is prevented.

23.1.2 Role of phytochrome in long day plants

Long day plants require a high ratio of pfr to pr for the formation of flowering stimulus. Such a high ratio of pfr to pr is attained at the end of a long day. If the night is too long, pfr reverts to pr or is destroyed and the flowering stimulus is prevented from being formed. When the night is interrupted by a flash of red light, pr is converted to pfr, there by raising the ratio of pfr to pr to a level which allows the flower stimulus to form.



23.2 FLOWERING HORMONES:

Gibberlic acid (GA) seems to play an important role in flowering. It may be directly involved in the formation of florigen.

GA's can substitute for long day requirements and also the cold treatment requirement in several species for its flowering. Auxins are also known to induce flowering in short day pine apple and they are used commercially for this purpose. They have also been found to be effective on long day plant like winter barley and *Hyoscyamus Niger*. Exogenous application of cytokinins is also known to induce flowering in many species, under non-inductive photoperiods including chrysanthemum and *Phorbitis nil*.

23.3 VERNALIZATION AND FLOWERING

It has been noticed that certain plants in addition to an appropriate photoperiod, require low temperature treatment during their early stages of life period for subsequent flowering in the later stages. It was found that winter wheat which is usually sown in winter and flowers in summer can be converted into spring wheat which is sown in spring and flower in summer, if slightly germinated seed are given artificial cold treatment. This conversion of winter variety of wheat into spring variety by low temperature or chilling treatment was termed as Vernalization by Lysenki (1928). Due to low temperature treatment the period of vegetative growth of the plant is reduced resulting into early flowering.

Chourad (1960) defined vernalization as acquisition or acceleration of the ability to flower by a chilling treatment. The optimum temperature for vernalisation is **0 to 5^{0}C**.

The phenomenon of vernalization was studied in several winter annuals Eg. Petkus winter rye (*Secale cereale*), some biennials Eg. Henbane (*Hyoscyamus niger*) and also in certain perennials Eg. Apples (*Pyrus malus*). All the above plants require a low temperature treatment for subsequent flowering. However certain plants such as Petkus winter rye, do not have an absolute cold requirement for flowering. In such cases, the cold temperature only shortens the time of flowering, where as in biennial strain of Henbane the vernalization treatment is absolute and they cannot flower without vernalization.

Perception of Cold Stimulus:

Apical meristem, embryos and actively dividing cells are the potential sites of vernalization. The common feature for vernalization is the presence of actively dividing apical meristems. Hence, vernalization treatment requires.

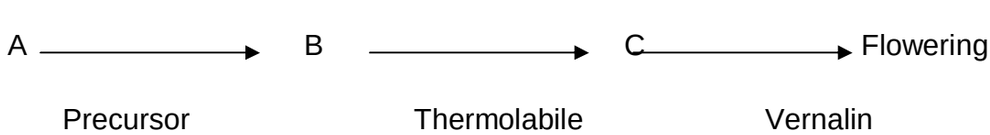
1. Actively dividing apical meristems.
2. Respiration substances such as carbohydrate.
3. Presence of oxygen.
4. Water (40 to 80%)

Presence of Floral Hormone:

The German botanist G. Melcher's (1939) suggested that low temperature induces the formation of a hormone, **vernalin** a hypothetical substance and has not been isolated so far. The cold stimulus can be transmitted from one plant to another by a graft union Eg. Henbane. Here, if a vernalized plant is grafted to an unvernalized plant the later also shows flowering.

Mechanism of vernalization: Hormonal Theory.

Lang and Melcher's (1947) suggested that a precursor A is converted into a thermolabile compound B during cold treatment. Under normal conditions B changes into a stable product (C Vernalin) which causes flowering. But at higher temperature B is converted into D and flowering does not take place due to devernalization. (fig.3). further experiments have shown that vernalin acts in association with the flower inducing stimulus, florigen in order to induce flowering.



Devernalization:

The effect of low temperature treatment for flowering is nullified if the plants are immediately given high temperature treatment. This phenomenon is called devernalization. The degree of devernalization decreases if the duration of the cold treatment has been longer. However, the devernalized plant can be vernalized again by subsequent cold treatment.

Vernalization and gibberellins:

Lang et al (1952) demonstrated that application of GA's can replace the low temperature requirement for vernalization in many plants. Eg. Henbane. This plant remains vegetative and retains its rosette habit during first growing season after passing through the winter period flowers in the next season. The GA's cause such plant to flower even during the first year.

23.4 IMPORTANCE OF VERNALIZATION:

1. Vernalization increases cold resistance in plants.
2. It reduces the vegetative period of development of plants and induces early flowering.
3. Winter varieties of crop plants can be converted into spring varieties by vernalisation.

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Lecture: 24

PLANT GROWTH REGULATORS

Growth of the plant has for long been believed to be due to the minerals absorbed from the soil and the food materials synthesized by the plant. It is now however recognized that the growth of the plant is very much regulated by certain chemical substances known as growth regulators. These substances are formed in one tissue or organ of the plant and are then transported to other sires where they produce specific effects on growth and development.

Philips (1971) defined growth hormones as a substance which is synthesized and is transported to other cells where in extremely small quantities influences development processes.

The plants are known to produce 5 classes of hormones namely auxins, gibberellins, cytokinins, abscisic acid and ethylene. A plant tissue may contain more than one of these growth regulators at the same time. The leaf primordial and developing seeds contain both auxin and gibberellins and in some plants ABA also. Both auxins and gibberellins cause stem elongation by different mechanisms while ABA and ethylene inhibits stem growth.Thus, two or more growth regulators may be similar in their action. When the effect is more than the sum of their individual effects it is called synergistic and when the action of two growth regulators is opposite it is called antagonistic. The final growth and development of the plant is the sum of total interactions of different growth regulators that are present in the plant.

24.1 AUXINS:

The idea of the existence of auxins in plants was for the first time conceived by Charles Darwin in 1881. He showed that coleoptile of canary grass could bend towards light when it is unilaterally illuminated. However the coleoptile failed to bend when its tip was covered with an opaque cap. Most of the knowledge about auxins comes from the work on oat (*Avena sativa*) coleoptile. Went (1926) demonstrated that coleoptile tips contain a substance capable of elongation of decapitated coleoptiles. He placed several freshly cut coleoptile tips on an agar block which was kept on a piece of inert material like glass. After several hours he cut the agar block into small cubes. He placed the agar cubes accentrically on decapitated coleoptile stumps for 2 hours in the dark. The effect of agar cube was similar to that of the tip as was shown by curvature of the coleoptiles(**Avena coliaptile curvature test**)

The first higher plant from which auxin could be extracted was maize kernels. It was identified as IAA. Indole Acetic Acid is the major auxin occurring in plants.

24.1.1 OCCURRENCE:

All the parts of the plant body produce auxin. However the major sites of auxin production are the **shoot tip**, **developing seeds** and **buds**. The amount of auxin present in different parts of the plant varies greatly. The amount is highest in the stem tip and coleoptile tip and decreased gradually down words.

Synthetic auxins:

There are a number of synthetic chemicals which are similar to IAA in their biological activity. However they do not occur in any plant. The important synthetic auxins are IBA (Indole Buteric Acid), NAA (Naphthalene Acetic Acid), 2, 4-D (2, 4 Dichloro Phenoxy Acetic Acid) and 2,4,5-T (2,4,5-Trichloro Phenoxy Acetic Acid).

24.1.2 BIOSYNTHESIS OF AUXINS:

Indole acetic acid (IAA) is synthesized from an amino acid Tryptophan in 3 different pathways. These 3 pathways have different intermediate compounds and the pathway is named after the intermediate compound produced as follows

(a) Indole Pyruvic Acid Pathway **(b)** Tryptamine Pathway and **(c)** Indole Acetol Doxime Pathway.

Indole Pyruvic Acid Pathway:

The first reaction involves transmission of Tryptophan to Indole Pyruvic Acid. The enzyme tryptophan amino transferase transfers amino groups (NH_2) from Tryptophan resulting in the formation of Indole Pyruvic Acid.

In second step Indole pyruvic acid is decarboxylated to form Indole acetaldehyde. The enzyme involved is Indole pyruvic decarboxylase. A molecule of CO_2 is removed. In the final step Indole acetaldehyde is oxidized to IAA by 2 enzymes namely Indole acetaldehyde dehydrogenase and Indole acedaldehyde oxidase.

Tryptamine Pathway:

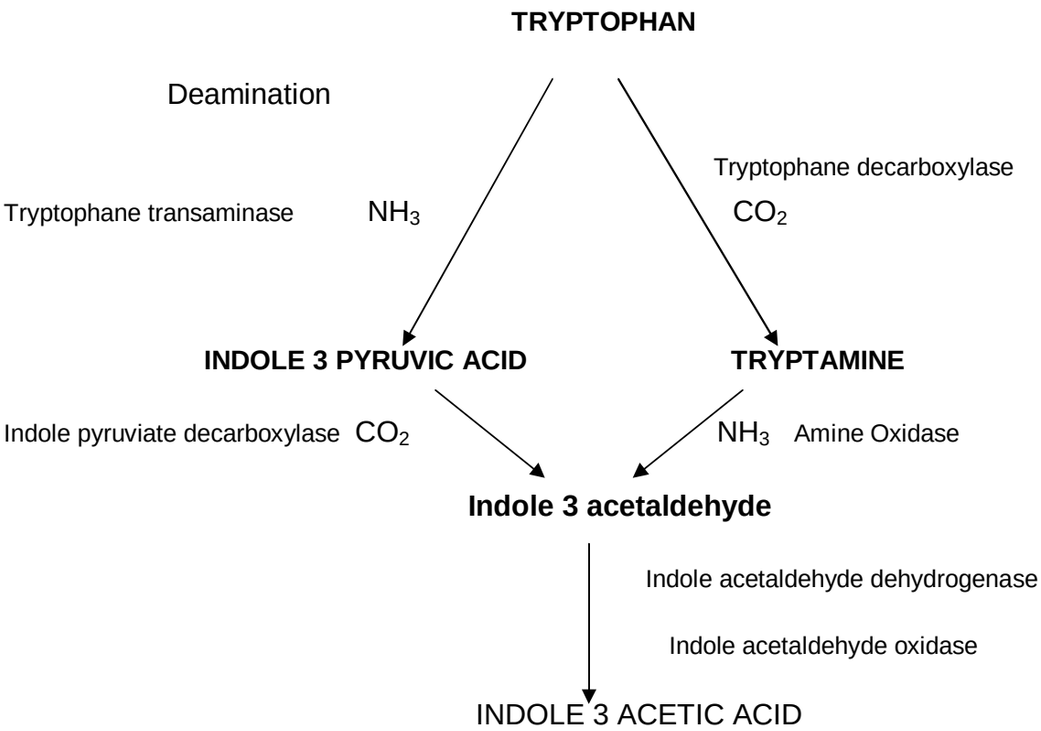
This pathway is of rare occurrence and was shown in tomato. Here tryptophan is first decarboxylated by the enzyme tryptophan decarboxylase forming tryptamine. Tryptamine then undergoes deamination forming indole acetaldehyde. The enzyme responsible for this reaction is amine oxidase. In the final step indole acetaldehyde is oxidized to IAA.

Indole Acetaldoxime Pathway:

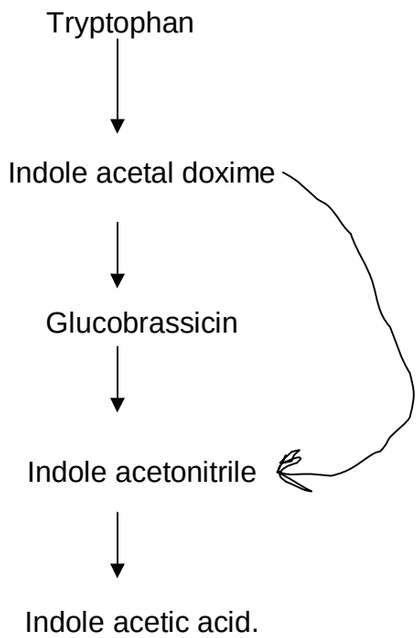
This pathway is characteristic of the members of the family Brassicaceae. In this pathway tryptophan is first converted to indole acetal doxime which in turn is

further converted to indole aceto nitrile directly or through an intermediary compound glucobrassicin. Indole aceto nitrile is converted to indole acetic acid. **Zinc** is essential for biosynthesis of auxin since it activates the enzyme **tryptophan synthatese**.

BIOSYNTHESIS OF AUXINS:



II. **Indole Acetal Doxime Pathway:**



TRANSPORT:

Auxin moves towards morphological basal end in stem cuttings. The movement of auxin is polar and basipetal. In roots it is polar but acropetal. In xylem it moves along the transpiration stream.

24.1.3 MODE OF ACTION:

It was observed that whenever auxin causes elongation of the coleoptile, the external medium in which the coleoptile was flooded, becomes acidic. The pH which was originally near neutral becomes decreased to 4.5. On this basis Hager, Menzel and Krans (1971) proposed **acid growth hypothesis of auxin action**. In this it was explained that the H^+ ions decreases PH value and presumed to break the acid labile bonds or activate wall hydrolyzing enzymes and render the cell was soft. This will create suitable conditions for cell elongation.

Auxin and cell elongation:

The primary physiological effect of auxin is to promote the elongation of cells which may be due to increasing osmotic pressure and permeability of cytoplasm to water and decreasing cell wall pressure.

Auxin stimulates the production of hydrolyzing enzymes like B-1, 3-gluconase, pectin methyl esterase and cellulase which soften cell wall and increase the plasticity resulting in reduction of wall pressure and cell elongation. Under the influence of auxin, cellulose synthetase increases and new wall material is synthesized within the cell wall resulting in extension or growth of the cell.

24.1.4 PHYSIOLOGICAL ROLE OF AUXINS:

A. Cell division:

Auxin has been found to be responsible for initiating and promoting cell division in certain tissues eg. Cambium. When ever wound is caused in the plant a swelling called callus is developed because of the proliferation of the parenchyma cells stimulated by auxin and a chemical substance traumatic acid. This can be put to practical use in **grafting** where the callus plays an important role in strengthening the union between stock and sion. Hence, during grafting of grapes, it was found that immersion of stock and sion in 0.1 percent of IAA resulted in quick growth of callus and success of graft union.

B. Root initiation:

The stem cuttings of some plants readily form adventitious roots when put in the soil. Adventitious root formation will takes place at the basal end of stem cuttings. Cuttings of such plants which do not readily root, form abundant roots when treated with auxins. Synthetic auxins like IBA, NAA are particularly very effective. In general cuttings of herbaceous plants readily respond to auxins while those of woody perennials like eucalyptus, mango and others fail to respond to auxin application.

In air layering of guava auxins like IBA at 500 ppm is used for root initiation.

C. Apical dominance:

The presence of apical bud causes a complete or partial inhibition of lateral buds. This is due to the presence of higher concentration of auxin at the apical bud, which causes a preferential movement of nutrients towards it.

In plants like sunflower, the main stem continues to grow and the lateral buds in the axils of leaves do not emerge and grow into branches. However when the tip of the main stem is cutoff or when it terminates into an inflorescence, the lateral buds emerge into branches. In either case the influence of the shoot apex on the lateral bud is lost. This phenomenon of inhibition of laterals by the shoot apex is termed as apical dominance.

In plants like potato and tomato apical dominance is weak consequently the apical growing point of the main stem fails to suppress the emergence of lateral buds. Such plants are therefore extensively branched and bushy.

D. Inhibition of abscission layer:

Abscission is a process of dissolution of the middle lamella and primary walls of the cells at the base of the petiole, pedicle or peduncle. Abscission refers to detachment of plant organs. It is a balance between the inhibition of auxin and promotion of substances like ABA, Ethylene etc several auxins (2,4-D, IAA, NAA) inhibits the abscission of both leaves and fruits.

Ex: application of NAA at 20 to 30 ppm twice at 15 days interval reduces flower and fruit drop in chillies and cotton, 2,4-D prevents defoliation in cabbage and cauliflower that often occurs during harvest.

E. Flower initiation.

Application of auxin inhibits flowering in several photoperiodically sensitive plants such as xanthium, soybean and others. But the exception is pineapple (*Ananas comosus*) a day neutral plant. This plant can be made to bloom promptly with the application of NAA or 2,4-D. However in this plant the effect of auxin is not direct but is mediated through ethylene formation.

Application of auxin also alters the sex ratio of flowers. In monoecious cucurbits increase the number of female flowers but the decrease the number of male flowers. Similarly in dioecious plants like cannabis, the male plants start producing female flowers when auxin is applied. Here again the effect of auxin is not shown to be direct but is mediated through ethylene formation.

F. Production of parthenocarpic fruits:

It is a general observation that in the absence of pollination and fertilization the ovary of the flower does not develop into the fruit, but the flower abscises and falls. However application of auxin causes development of ovary into the fruit in several plants such as tomato, brinjal and others. Such fruits are seedless as these have developed without the normal process of fertilization these are known as parthenocarpic fruits. The presence of large number of seeds in a fruit lowers its commercial value in the canning industry.

G. Eradication of weeds:

Plant roots are extremely sensitive to auxins. Very high concentration of auxins over stimulates growth promoting activities of root cells resulting in distorted roots with blocked sieve tubes. The roots ultimately decay and the plant is killed. 2,4-D and 2,4,5-T are effective weedicides at higher concentration of 1 to 3 percent . **2,4-D** is selective weed killer. It is highly toxic to **broad leaved plants** or dicotyledons while relatively non toxic to narrow leaved plants or monocot.

H. Growth in thickness:

The stem of dicots and gymnosperms not increase in length but also in thickness. Increase in thickness of the stem and root is due to radial growth. The process is termed as secondary growth.

I. Vascular differentiation:

Not only the activation of cambial rings but also the differentiation of cambial derivatives into xylem and phloem is also under the control of hormones. Interaction of both auxin and GA is involved in this.

J. Prevention of lodging:

Naphthyl acetamide when applied on the base of oats and flax, they grow stiff, woody and erect. Thus lodging in these crop plants is prevented.

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Lecture:25

PLANT GROWTH REGULATORS

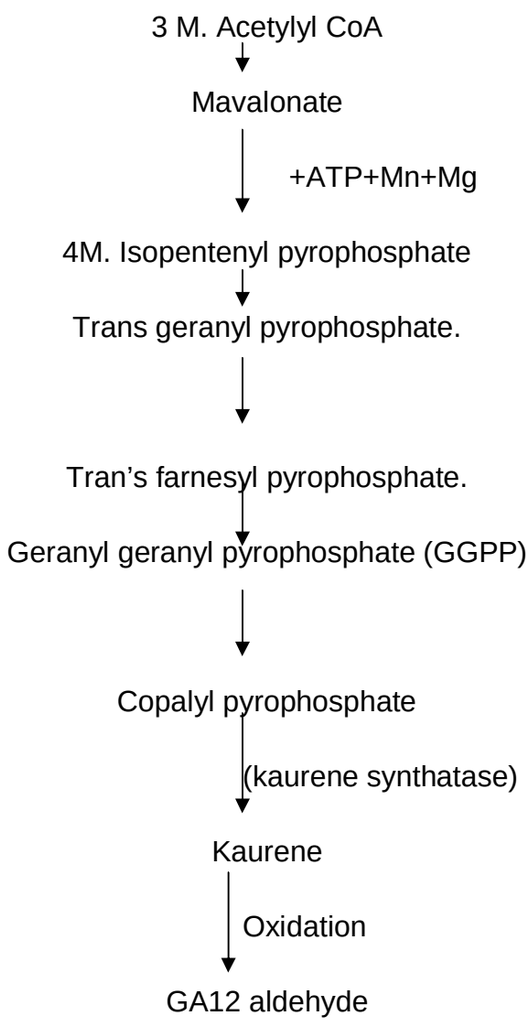
GIBBERELLINS

The discovery of Gibberellins was quite accidental. Japanese worker Kurosawa (1926) in Japan while conducting experiments on rice disease caused by *Gibberella fujikuroi (causal organism for foolish seedling of rice or bakane disease)* observed that the fungus caused excessive growth in rice. He applied the fungal extracts to intact healthy plants and observed enhanced growth. Later Yabuta and Sumuki (1938) named the active principle as gibberellin. Further it was purified, crystallized and named as gibberellic acid (Curtis and Cross 1954). Now gibberellins are designated as GA_1, GA_2 and so on. The common gibberellic acid is GA_3. At present 112 types of gibberellins are known.

25.1 Occurrence and Site of synthesis:

Gibberellins are synthesized in the young leaves (major site), shoot tip, root tip and the developing seeds.

25.2 Bio synthesis:



Acetyl co A is the precursor for the biosynthesis of gibberellins. Three molecules of acetyl co A are linked together to form a molecule of mevalonic acid. Mevalonic acid in turn is activated in the presence of ATP, Mn and Mg and is converted to isopentenyl phosphate (IPP). This is a 5 carbon compound. Four molecules of IPP undergo stepwise condensation, first to Trans geranayl pyrophosphate (GPP) then to trans farnesyl pyrophosphate (FPP) and finally to form a diterpene called geranyl geranyl pyrophosphate (GGPP). This is 20 carbon compound. This GGPP is converted to kaurene. The conversion of GGPP to kaurene is carried out by the enzyme kaurene synthetase in two steps.

First GGPP is converted to copalyl pyrophosphate. In the second step copalyl pyrophosphate in turn is converted to kaurene. Kaurene undergoes oxidation and a series of reactions resulting in the formation of gibberellins.

Transport

Transport of gibberellins is passive and non polar. Gibberellins move both in xylem and phloem and vice versa through vascular ray cells.

25.3 Mode of action:

There are several hypotheses to explain the mechanism of GA in the plants.

1. Increase in the endogenous auxin content:

Whenever GA causes cell enlargement, the effect is not considered to be direct. The effect is indirectly mediated through formation of auxin, in turns is held responsible for the cell elongation.

Gibberellin has been shown to cause **synthesis of amylase in barley aleurone cells**. This enzyme converts starch to reducing sugars resulting in an increase of osmotic pressure, causing entry of water into the cells and cell enlargement.

25.4 PHYSIOLOGICAL EFFECTS:

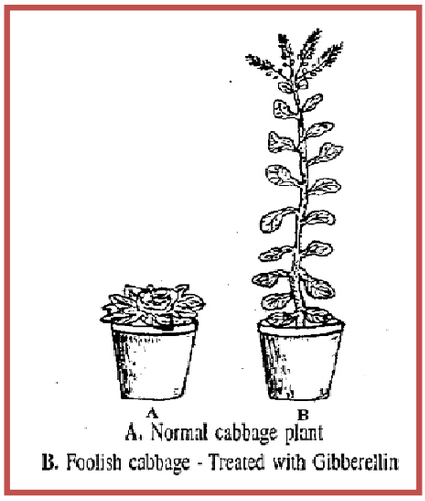
A. Stimulation of stem growth:

The most important effect of GA is the stem elongation when GA is applied the stem elongates markedly. As a result such plants grow taller. GA caused stem elongation has the following characteristic features (1) enhanced stem growth is not due to increased formation of nodes and internodes but results from rapid elongation of internodes. Therefore GA treated plants do not differ from control plants in the number of nodes and internodes. Elongation of internodes is due to both cell division and cell elongation. Younger internodes respond better than older ones and plants grown in light respond better to GA than those grown in the dark. However, not all

plants respond equally to GA application. It is only the genetic dwarf and rosette plants which show marked stem elongation.

Genetic dwarfs:

From the field grown maize four mutants were isolated. These mutants grow only up to about one fourth the heights of normal tall varieties. When gibberellin is applied, the dwarf plants respond readily and show marked stem elongation. These become comparable in height to corresponding tall varieties. Here the degree of stem elongation is proportional to the concentration of GA that is applied.



A. Normal cabbage plant
B. Foolish cabbage - Treated with Gibberellin

B. Bolting:

Production of floral axis is called bolting. Bolting and flowering are induced normally after photo induction or vernalisation. Bolting however can be induced without vernalisation by the treatment of the plant with gibberellins.

Many plants require a period of low temperature for flowering. Application of GA replaces the vernalization (0-5^{0}C) requirement for the flowering of carrot, beetroot, chicory and others. Vernalization or low temperature requirement is usually met with when the plants pass through natural winter. However this low temperature requirement can be completely overcome and plants can be made to flower in high temperatures by applying GA.

Therefore low temperature requirement of plants can be replaced with GA.

C. Flowering in long day plants:

Gibberellins promote flowering in long day plants under un favorable SD conditions. Ex: Niger.

D. Parthenocarpic fruits:

Gibberellins have been found to be more effective than auxins in causing parthenocarpic development of fruits in plants like tomatoes, apples, pears and stone fruits.

Gibberellin application promotes panicle exertion. Generally 30% of the panicle is covered by leaf sheath. Application of GA + Brassinosteroids is practically used in commercial seed production of Rice.

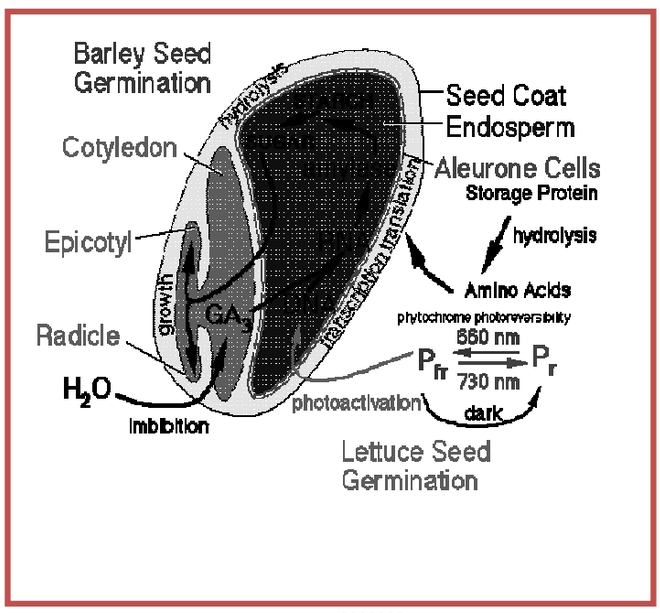
E. Breaking of dormancy:

Gibberellins are effective in breaking the dormancy in potato tubers and in tree buds in winter.

In potato the tubers remain dormant for weeks after harvest. However when GA is applied the buds sprout soon after the tubers are harvested. This will be useful to use the freshly harvested tuber for sowing. The seed material has to be dipped in 0.5 to 1.0 g of GA /lit of water.

ANNEXURE

Gibberellin has been shown to cause synthesis of amylase in barley aleurone cells. This enzyme converts starch to reducing sugars resulting in an increase of osmotic pressure, causing entry of water into the cells and cell enlargement. GA3 is also known to increase permeability of aleurone cells to sucrose. It increases the activity of membrane synthesizing enzymes. Synthesis of phospholipids is also increases due to GA application. The gearing up of all these metabolic activities results in cell elongation.



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Lecture:26

PLANT GROWTH REGULATORS

CYTOKININS

Skoog and his coworkers discovered cytokinins when they were trying to identify a compound to initiate and sustain the proliferation of cultured tobacco pith tissue. Crystals of a cell division inducing substance was later isolated for the first time by **Miller,** from an autoclaved herring sperm DNA in 1951 and named it as **Kinetin**. The liquid endosperm of coconut (coconut milk) is also found to be rich in cell division causing factors. **Letham** (1963) extracted, purified and crystallized cytokinin from immature kernels of maize and named it as **zeatin**.

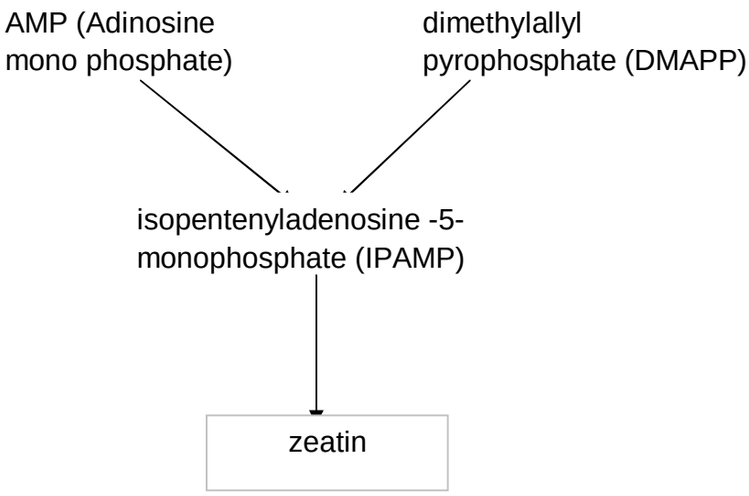
26.1 OCCURRENCE:

Naturally occurring cytokinins are N^6- substituted adenine derivatives. Usually **Zeatin** is the most abundant naturally occurring free cytokinin. There are also synthetic cytokinin compounds that have not been identified in plants, most notably of which are the **diphenyl urea type cytokinins**, such as **thidiazuron**, which is used commercially as a defoliant and an herbicide.

Cytokinins, occur freely and also as a component of RNA of plants, microorganisms and animals. In higher plants root tips, shoot tips, developing fruits, xylem sap and germinating seeds are rich sources of cytokinins. Root tips synthesize cytokinins and transport them through the xylem to all parts of the plants. This might explain their accumulation in young leaves, fruits and seeds in to which xylem transport occurs.

26.2 BIOSYNTHESIS:

The biosynthetic pathway of free cytokines is not completely understood. There are two methods in which they may be produced. The first is the direct pathway, involving formation of Isopentenyladenosine-5-monophosphate (IPAMP) from AMP and dimethylallyl pyrophosphate (DMAPP), to form zeatin-type compounds.



Another possibility is that they may be released by the **hydrolysis of tRNA**, first to mono nucleotides and then to free cytokinins. In spite of extensive effort having been focused on these pathways information is still highly fragmented.

TRANSPORT:

When cytokinin is applied to leaves and stems, the hormone does not move and the effect is localized. Cytokinin is carried passively along the transpiration stream in xylem from root. It moves in phloem in a basipetal polar direction in very small quantities.

26.3 MODE OF ACTION:

Cytokinin is a structural component of transfer RNA molecule. They may help in binding of mRNA with tRNA - amino acid complex during protein synthesis. Cytokinins increase the synthesis of nucleic acid by increasing the enzyme t RNA synthatase and decrease the degradation by reducing the activity of ribonuclease. Cytokinin increases the incorporation of phosphorous in to nucleic acids and adenine into RNA.

26.4 PHYSIOLOGICAL ROLE:

- **Cell division**

Cytokinins are known to be regulators of cell division in mature cells. The most important effect of cytokinins is stimulation of cell division in excised tissues. The number of cell divisions increases proportionally to the concentration of added cytokinin when auxin is not limiting. Cytokinins alone does not promote cell division. When both auxin and cytokinins are added together, cells divide rapidly and the callus tissue grows.

- **Morphogenesis**

Root and bud differentiation

Cytokinins in interaction with auxins control morphogenesis. The cells of tobacco pith do not either grow or differentiate when only auxin or only cytokinin is added to the medium. However when the medium contains both auxin and kinetin in the ratio of 10:1 pith cells grow and forms a mass of unorganized cells (callus).

If the ratio of auxin to cytokinin is more in the medium, a number of roots are initiated from the callus. If the ratio is less (which means more cytokinins than Auxins) a number of shoot buds are initiated.

- **Anti Senescence hormone (Richmond - Lang effect)**

Cytokinins delay senescence. Generally, protein and chlorophyll content of the leaf decreases with the increase in age. Thus, when leaf becomes old, it turns in to yellow, become senescent and finally shed of. Senescence of leaves can be delayed by application of kinetin. Cytokinins delay senescence by increased synthesis of proteins.

The delay of senescence of leaves and other organs of the plants by cytokinins is called as **Richmond - Lang effect.**

In an experiment, one of the two primarily opposite leaves of a bean plant was treated with Benzyl adenine. This treatment accelerated senescence of untreated leaf. This is because of mobilization of organic metabolites and minerals from untreated leaf to cytokinin treated leaf because of cytokinin acts as mobilizing centers.

Retardation of senescence of vegetables can be achieved by cytokinins. Green vegetables like cabbage, lettuce and celery deteriorate rapidly after harvest. Post harvest spray of Benzyl adenine at 10 to 40 ppm or post harvest dip of 10 ppm increase shelf life of these vegetables.

- **Promotion of lateral bud growth**

Application of cytotinins reduces apical dominance. The action of cytokinin is antagonistic to that of auxin in apical dominance. The lateral buds of intact plants which otherwise remain arrested; can be made to grow by applying kinetin. It may be due to the differentiation of vascular tissue in the presence of cytokinins.

The pathogen *Corynebactrerium facians* causes a disease called **Witches broom** in many plants. This symptom is characterized by loss of apical dominance and emergence of numerous lateral branches which give the appearance of a

broom. This effect is due to the secretion of cytokinin namely isopentenyl adenine by the pathogen.

- **Breaking of dormancy**

Cytokinins can replace the red light (660 nm) requirement in seed germination of lettuce and tobacco. Lettuce seeds require the presence of red light for germination in addition to moisture, air and suitable temperature. However the seeds can be made to germinate in the dark by applying Kinetin. Thus, Kinetin replaces the red light requirement for germination.

In cocklebur, each fruit (but) contains two seeds which are of unequal in size. The lower one in largest and germinates while the upper seed is dormant. Here the dormancy is due to the presence of germination inhibitors. This dormancy is over come by the application of kinetin.

- **Cell enlargement**

Cortical cells of tobacco root were observed to enlarge four times of their normal size in the presence of kinetin.

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Lecture:27

PLANT GROWTH REGULATORS

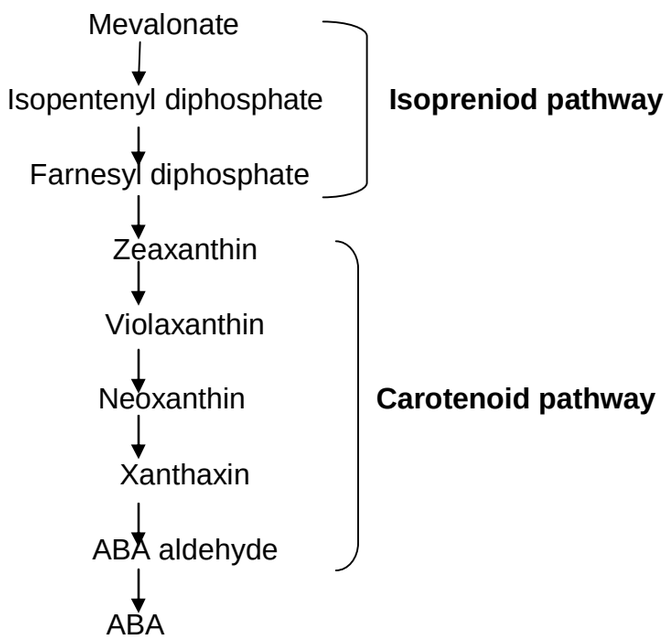
ABSCISIC ACID (STRESS HORMONE)

27.1 Occurrence: the plant growth regulator ABA is one of the wide spread and naturally occurring inhibitor found in plants. Addicot and his colleagues (1964) isolated this abscission causing compound from cotton bolls and named it as abscisin I & abscisin II. It is now known that ethylene is the hormone that triggers abscission and that ABA induced abscission of cotton bolls is due to ABA's ability to stimulate ethylene production. In higher plants ABA occurs in all parts of the plant body. It has been reported from the leaves of birch (*Betula sps*) and tubers of potato. ABA is found in all parts of the seed namely the seed coat, embryonic axis, cotyledons and endosperm.

27.2 Biosynthesis:

It is a sesquiterpenoid (15-carbon) which is partially produced via the mevalonic pathway in chloroplasts and other plastids.

ABA is synthesis in plants involves two pathways (1) carotenoid pathway (2) Mevalonic acid pathway or Isoprenoid pathway.



Site of synthesis

All parts of the plants such as stem, root and leaves. Fruits and seeds are also capable of ABA synthesis.

Transport

ABA is transported by both the xylem and phloem, but it is normally much more abundant in the phloem sap.

27.3 Mode of action

ABA is involved in the short term physiological effects (e.g. stomatal closure), as well as long term developmental processes (e.g. seed maturation).Rapid physiological responses of ABA frequently involve alteration in the fluxes of ions across membranes and may involve some gene regulation as well.

Mode of action of ABA in causing various physiological effects can be seen in three different ways

1) ABA brings about changes in membrane permeability for different ions (Like

K^+, Ca^{++} etc.) . It plays a role in stomatal closure.

2) ABA inhibits DNA & RNA synthesis (transcription) finally leads to senescence.

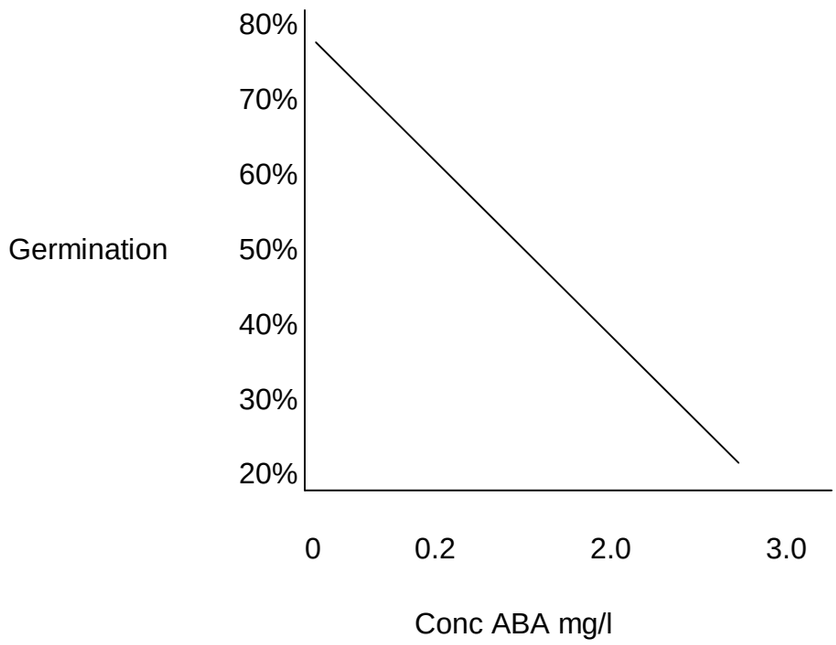
3) It inhibits translation (Protein synthesis) thus formation of enzymes is blocked.

Germination process is affected ultimately leads to dormancy.

27.4 <u>PHYSIOLOGICAL ROLE</u>

A) Seed dormancy:

Application of ABA inhibits seed germination in several species.



Similarly seeds which are dormant are shown to contain ABA.

Seeds of apple remain dormant and fail to germinate till they are exposed to a period of stratification. such seed show the presence of ABA. When the seeds are stratified the ABA content falls with a corresponding increase in GA content. Thus, it can be concluded that the seed dormancy is controlled by GA-ABA balance at least in some species.

ABA helps in inhibiting precocious germination and vivipary. This is very important because dormancy caused by ABA do not allow the seed to germinate while it is still on its mother plant.

B. Bud dormancy

In woody species, dormancy is an important adoptive feature in cold climates. When a tree is exposed to very low temperatures in winter it protects its meristems with bud scales and temporarily stops bud growth. Bud dormancy in Acer, betula and other temperate tree sps. This is accompanied by build up of ABA within these plants.

C. Effect of stomata

Application of ABA causes rapid closure of stomata. The stomatal aperture progressively decreases with the concentration of ABA.

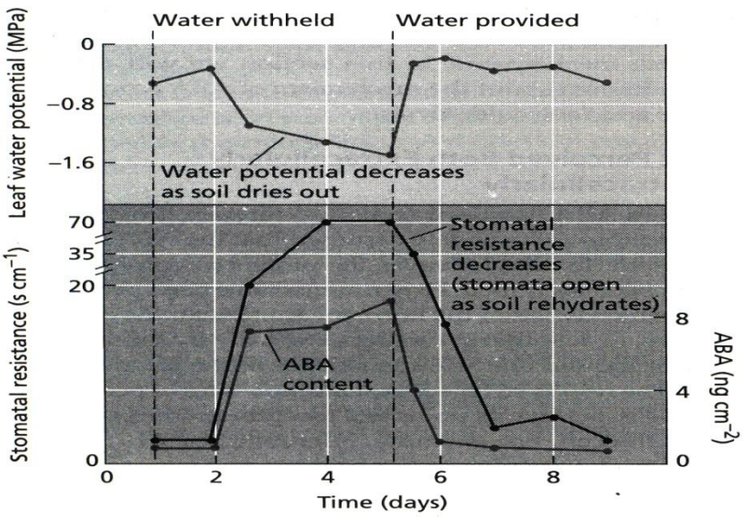


FIGURE Changes in water potential, stomatal resistance (the inverse of stomatal conductance), and ABA content in maize in response to water stress. As the soil dried out, the water potential of the leaf decreased, and the ABA content and stomatal resistance increased. The process was reversed by rewatering. (After Beardsell and Cohen 1975.)

*FIGURE IS FROM REFERENCE 6

ABA accumulates in higher concentration in wilting leaves. This accumulated ABA closes stomata. ABA might inhibit the formation of enzymes which are responsible for the conversion of starch into sugar and formation of organic acids. It reduces the osmotic concentration and causes closure of stomata.

D. Senescence

ABA is quite effective in promoting senescence of excised leaf disks of both monocots and dicots. Although leaf disks are affected, when sprayed on the corresponding intact leaf, ABA is not effective even at higher doses in inducing senescence.

E. Flower initiation

ABA induces flowering in SD plants and inhibits the same in LD plants. Here the hormone inhibits vegetative growth and causes apical bud dormancy. In such plants onset of dormancy precedes flowering. Therefore, effect of ABA on flowering is indirect.

F. Antagonism

ABA inhibits GA stimulated growth in various forms. Therefore ABA is known as Antigibberellin.

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Lecture:28

ETHYLENE

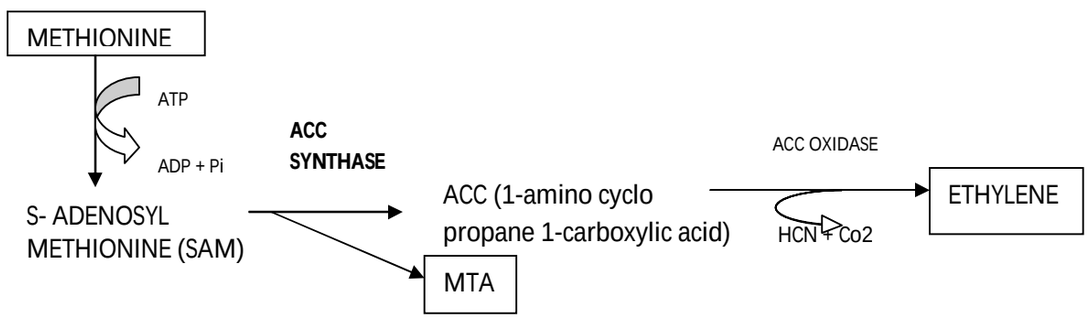
Neljubow (1901) a Russian plant physiologist was the first to show the importance of ethylene present in the illuminating gas as a growth regulator of plants. He observed that dark grown pea seed lings growing in the laboratory (illuminated with coal gas) exhibited symptoms that were later termed as *triple response*: reduced stem elongation, increased lateral growth, and abnormal horizontal growth. Denny (1924), reported that ethylene is highly effective in inducing fruit ripening. **Gane** (1934) established that ethylene is produced by the apple fruits during ripening in storage.

28.1 Occurrence

In higher plants, all most the parts of the plant body produce ethylene.In general meristematic regions and nodal regions are most active in ethylene biosynthesis. However, ethylene production also increases during leaf abscission and flower senescence, as well as during fruit ripening. This is otherwise called as phytogerontological hormone .

28.2 Bio-synthesis

The amino acid Methionine is the precursor of ethylene, and ACC (1-amino cyclo propane 1-carboxylic acid) serves as an intermediate in the conversion of methionine to ethylene. Methionine activated by ATP gives rise to S-adenosyl Methionine (SAM). This reaction is mediated by enzyme Methionione adenosyl transferase. In the next step, SAM breaks into 5'-methyl thio adenosine (MTA) and amino cyclo propane carboxylic acid (ACC). This reaction is carried by the enzyme ACC synthase. ACC is oxidized to ethylene with the release of HCN (Hydrogen cyanide) and Co_2.



Amino ethoxy vinyl glycine (AVG), and Amino oxy acetic acid (AOA) block the conversion of SAM to ACC. Silver Nitrate and Silver thio sulphate are the specific inhibitors of ethylene action.

Ethylene Transport

Ethylene being a gas can easily diffuse into plant tissues through the intercellular spaces. From ripening fruits, ethylene diffuses out into the atmosphere through the cut end of pedicel and fruit surface.

28.3 MODE OF ACTION:

There are several theories to explain the mechanism of action of ethylene.

Membrane permeability

Ethylene is considered to dissolve in cell membranes altering their permeability. Ethylene is highly soluble in lipids which are constituents of cell membranes.

Another hypothesis is by regulating auxin metabolism. Ethylene treatment results in a reduction in the content of diffusible (free) auxin. This may result from (1) decreased synthesis (2) decreased transport and (3) increased binding. Ethylene has been shown to inhibit transport of auxin from the site of production to the site of action.

28.4 PHYSIOLOGICAL ROLES (Includes both positive and negative effects)

A. Fruit ripening

Broadly fruits can be classified into two types on the basis of their respiratory pattern during ripening. In some fruits like apple and banana as the fruit matures and attains its maximum size, the rate of respiration decreases and becomes very low. After the fruit is harvested and stored for ripening, there is a great increase in the rate of respiration and the rise continues till it attains a sharp peak. This is called climacteric peak and the fruits are called climacteric fruits. In climacteric fruits ripening occurs even after harvesting. The climacteric rise is soon followed by a sharp decline.

The non climacteric fruits like grapes and lemon, the respiratory rate gradually decrease after the fruit is harvested without showing any abrupt rise. The peak respiratory rate in climacteric fruits usually corresponds to peak ethylene production.

Application of ethylene hastens ripening of climacteric fruits such as banana, mango, apple and tomato. This is being commercially employed. In non climacteric fruits such as lemon and orange ethylene application does not hasten ripening however rate of respiration increases greatly.

B. Abscission and senescence

Ethylene promotes both abscission and senescence of flowers. The flowers of orchids and roses the most sensitive to externally applied ethylene. Ethylene also

promotes leaf abscission. In general older leaves are more sensitive to ethylene and abscise faster than younger ones. Older leaves produce more ethylene than younger ones. This is probably responsible for abscission of older leaves.

C. Roots on stem cuttings

Application of ethylene promotes callus formation and initiation of adventitious roots on the stem cuttings. Some times adventitious roots may arise on the stem of intact plant as well.

D. Root and shoot growth

Ethylene inhibits linear growth of the stem and root of dicotylidons. The effect increases with increasing concentration.

E. Flowering and sex expression

Application of ethylene causes flowering in pine apple and shift the sex ratio of flowers towards femaleness in several cucurbits and cannabis.

F. Epinasty

Ethylene causes swelling of cells on the upper part of the petiole of the leaf resulting in drooping of leaves (down ward curvature). This is termed as epinastry. It is best exhibited in leaves of tomato, potato and pea etc.

G. Thinning in apple

Thinning of fruits in apple eliminates biennial bearing and also improves fruit size and quality. Application of ethephon at 100 to 300 ppm reduces fruit set. In cotton also ethylene induces this fruit thinning.

H. Exudation of sap and latex

When ethereal is applied to rubber plants, flower of latex continues for a longer duration Etherel probably prevents coagulation of late and consequent blocking of laticiterious ducts.

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Lecture:29 **PLANT GROWTH REGULATORS**

29.1 NOVEL PLANT GROWTH REGULATORS

A) JASMONATES: Jasmonic acid (JA) and its methyl ester (MEJA) occur in several plants and also in the oil of jasmine.

a) JA and MeJA inhibit the germination of non dormant seeds and stimulate the germination of dormant seeds.

b) JA plays a role in the formation of flowers, fruit and seed. It is suggested by the relatively high levels of this compound in developing plant reproductive tissues.

c) Reported a role in insect and disease resistance of plant.

d) JA stimulates tomato and apple fruit ripening.

B) BRASSINO STEROIDS (BRs) : Brassinolide, a potent plant growth stimulator, was the first BR isolated; it was discovered in rape (*Brassica napus*) pollen in 1979.So far, more than 40 brassinolide analogues, collectively known as brassinosteroids, have been identified and characterized from many different plant species.

- Immature seeds and pollen contain the highest concentrations of brassinosteroids.
- When applied exogenously to intact plants at micromaolar or nanomolar concentrations BRs can induce a variety of physiological responses, including seed germination, pollen tube growth, stem elongation, leaf unrolling and bending, ,vascular differentiation, induction of ethylene biosynthesis, altered gene expression, and stress response modulation.
- They help to Increase flowering fruit set and yield
- Foliar spray of 0.3 ppm BR at panicle initiation and flowering stage increases yield in rice

C.SALICYLIC ACID: Salicylic or ortho hydroxy benzoic acid belongs to a diverse group of plant phenolics. The interest in salicylic acid began with the discovery that this compound is a natural trigger for the metabolic explosion which raises the temperature of the thermogenic inflorescences of Arum Lillie.

- SA increase flower longevity by inhibiting ethylene biosynthesis by blocking the conversion of ACC to ethylene.
- SA regulates some aspects of disease resistance.

D. TRIACONTANOL: Saturated primary alchohol isolated from shoots of alfalfa. Response is very rapid in increasing growth. Enhanced growth in rice and maize is reported.

29.2 GROWTH RETARDANTS

The term growth retardant refer to the chemicals that slow down cell division and cell elongation of shoot tissue and regulate plant height physiologically without formative effects. They do not occur naturally in plants.

Examples:

- AMO 1618 ,
- CCC (Chloro choline chloride) (2-Chloroethyl – trimethyl ammonium chloride)
- Chlormequat chloride (Cycocel)
- Alar or B9
- paclobutrazol
- Mepiquat chloride,

29.3 GROWTH INHIBITORS

Growth inhibitors suppress the growth of plants. ABA and ethylene are called as natural growth inhibitors. They bring about certain formative changes in plants. There are synthetic growth inhibitors also.

Examples:

- Malichydrazide (MH)
- 2,3,5-T or Triiodo benzoic acid (TIBA)

29.4 COMMERCIAL APPLICATION OF PLANT GROWTH REGULATORS IN AGRICULTURE AND HORTICULTURE:

A) AUXINS

a) IBA (@250 ppm) and NAA were found to increase root development in the propagation of stem cuttings.

b) 2,4-dichlorophenoxy acetic acid (2,4-D) stimulates excessive uncontrolled growth in broad leaf plants for which it is used as a herbicide.

c) Application of NAA (Napthalene Acetic Acid) reduces flower and fruit drop in Mango.

d) NAA application brings uniform flowering and fruit set by inducing ethylene formation in Pineapple.

e) NAA application @ 10-100 ppm during fruit setting period controls boll shedding in cotton crop.

B.GIBBERELLINS:

a) GA is used extensively on seedless grape varieties to increase the size and quality of the fruit. Pre- bloom spray of 20 ppm induces rachis of the fruit cluster to elongate. This creates looser clusters that are less susceptible to disease during the growing season.

b) GA is used to increase the yield of barley malt and to decrease the time required for this process to occur. Application of GA to germinating barley supplements the endogenous content of this hormone and accelerates the production and release of hydrolytic enzymes. They can easily degrade the stored carbohydrates.

c) Foliar spray of $GA_{3,}$ at 100 ppm during panicle initiation stage enhances the panicle exertion and increases seed weight and yield in hybrid rice.

d) GA has also has been used to control flower sex expression in cucumbers and squash. GA application tends to promote maleness in these plants.

e) Gibberellic acid is also applied to citrus crops, though the actual use depends on the particular crop. For example GA_3 is sprayed onto oranges and tangerines to delay or prevent rind-aging, so that fruit can be harvested later without adverse effects on rind quality and appearance. For lemons and limes, GA_3 synchronizes ripening and enhances fruit size.

f) Gibberellic acid is used extensively to increase the sucrose yield of sugarcane. Sugarcane, a normally fast-growing C_4 member of the Poaceae (grass) family, is sensitive to cooler winter temperatures, which reduce internode elongation and subsequent sucrose yield. The adverse effects of cooler temperatures can be counteracted by the application of GA_3.

C.ETHYLENE:

a) Because ethylene regulates so many physiological processes in the plant development it is the most widely used plant hormones in agriculture. Auxins and ACC can trigger the natural biosynthesis of ethylene and in several cases are used in agricultural practice.

b) Because of its high diffusion rate, ethylene is very difficult to apply in the field as a gas, but this limitation can be over come if an ethylene releasing compound is used. The most widely used such compound is ethephon or 2-chloro ethyl phosphonic acid (**CEPA)**(trade name: **ethrel**).

c) Ethrel @ 100-250 ppm sprayed at 2-3 leaf stage induce femaleness in cucumber and melons.

d) It helps in degreening of citrus and banana which increases its market acceptability.

e) Storage facilities developed to inhibit the ethylene production and promote preservation of fruits have a controlled atmosphere of low 0_2 concentration and low temperature that inhibits ethylene biosynthesis. A relatively concentration of $C0_2$ (3-5%) prevents ethylene action as a ripening promoter.

D) OTHER GROWTH REGULATORS:

- AMO 1618 (a quaternary ammonium salt) is used in the cultivation of ornamental plants and causes a bushy shape and a sturdy growth of the treated plants.
- paclobutrazol : Reduces the problem of biennial bearing in Mango
- Mepiquat chloride, Chlormequat chloride (Cycocel) : used in ornamental plants for shorter internodes and thicker stems (used in poinsettias), it also prevents lodging and increases tillering in cereals.
- Malichydrazide (MH): prevents premature sprouting of onion and potato
- 2,3,5-T or Triiodo benzoic acid (TIBA): Increases flowering in chrysanthemum

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Lecture-30

SENESCENCE

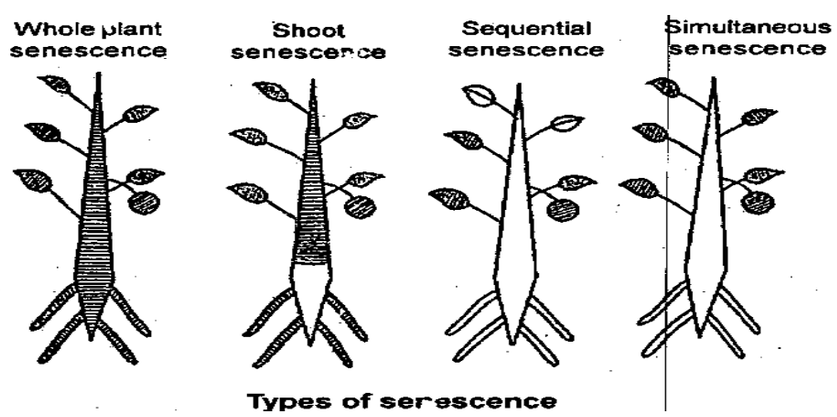
30.1 Definition: The term senescence' is derived from a Latin word "**Senescere**" which means to grow old'. The terms Senescence, Programmed Cell Death (PCD)', Apoptosis' and Ageing' are often used synonymously in plant or animal systems. All these terms generally refer to death of cells, organs or organisms.

In the life cycle of higher plants when they have reached a certain stage of maturity, they senesce and die. It should be clarified that the ageing is not the same as senescence. '**A degenerative and irreversible change in a plant which leads to death**' is called **senescence** where as **ageing** is '**the process of attaining maturity with the passage of time**'.

30.2 CLASSIFICATION OF SENESCENCE (TYPES OF SENESCENCE):

Depending upon the part of the plant in senescence, **Leopold** (1961) has classified senescence in to following four categories.

A. **Overall senescence**: (whole plant senescence): In this kind of senescence, there is senescence and death of the entire plant, which usually takes place at the end of reproductive phase.
   Eg: Cereal crops like maize, wheat and rice and also in mustard and cabbage
B. **Progressive senescence**: In normal development of most annual plants, there is progressive senescence, where the oldest leaves senesce and die first. The senescence moves from leaves to the stem and then to under ground parts.
   Eg: Tobacco.
C. **Top senescence** (Shoot senescence) Here senescence and death of all above ground parts occurs, while the under ground root portions survive and give rise to new buds in the next season.
   Eg: Sugar beet, Banana, Ginger
D. **Deciduous senescence (**simultaneous senescence**)**: In this kind of senescence, all leaves senesce and die, leaving the stem and roots alive as in gulmohar (*Delonix regia*) and Raavi (*Ficus religiosa*), Eucalyptus.



Types of senescence

The occurrence of senescence differs both in their causes and in its nature. Senescence of the entire plant after a single reproductive cycle is called **monocarpic senescence.** Senescence may be delayed when flowers and fruits are removed. Some monocarpic plants like *Agave americana* generally live and grow for many years before flowering. They die when they produce fruits. Thus senescence is an irreversible one. On the other hand in tobacco plant older leaves senesce as the plant grows. But the senescence can be reversed when the apical meristem of the plant is removed.

30.3 THEORIES OF MECHANISM OF SENESCENCE:

30.3.1 Nutritional theory: Molisch (1920) suggested that senescence was caused by nutritional deficiency. Different parts of the plants compete for nutrition. Fruit or growing tip for example, might form stronger sinks for translocation and thus accumulate much of nutrients, that the older leaves would be starved. This triggers off the senescence process in the older leaves and they start acting as a nutrient source for younger structures. One of his observations is that, if the fruit, seed or growing tip is removed, senescence is greatly delayed in the leaves. However, nutritional theory is not applicable to many situations. It is not possible to retard senescence in annual plants that have flowered or fruited even by application of fertilizers. Dioecious plants, which bear only male flowers and require no additional nutrients for fruit formation also undergo senescence.

30.3.2 Hormonal theory: It is observed that senescence is initiated as a result of change in hormonal content of the organ. Some believe that a hormonal signal is sent from developing fruits to leaves and other vegetative parts, where it triggers senescence. It is now well known that ABA produced in the developing fruits and seeds induce senescence in the leaves.

Nooden and Leopold (1978) proposed a term "**Death hormone**" for the causative agent of senescence in monocarpic species. The death hormone is a chemical substance which is supposed to be produced in developing seeds and translocated to vegetative parts through xylem and initiates senescence in leaves. This hypothesis is based on the observations that preventing flowering or fruiting retarded senescence in many cases.

30.3.3 Alternations in nucleic acid and protein contents with senescence and usually it will decline in senescing plants (Carr and Pate, 1967)

30.3.4 Increase in enzymatic activities of ribonuclease and proteases with the onset of senescence in plant (Martin and Thimann, 1972)

30.3.5 Structural alterations particularly evidence of **deteriorative changes in membranes** and organelles (Poovaiah and Leopold , 1973)

30.3.6 Increased **accumulation of free radicals** with ageing (Choudhri, 1988)

No hypothesis is fully satisfactory or complete and various criticisms can be put forward for and against of these hypothesis.

30.4 METHODS OF PREVENTING LEAF AND FLOWER SENESCENCE:

All the plants do not exhibit same response to growth hormones. Cytokinins appear to be more effective in delaying senescence in many herbaceous plants. GA's are effective in preventing senescence of ash (Fraxinus) because the endogenous GA content of the leaves decrease during leaf senescence. Auxins (IAA and 2,4-D) have been found to retard senescence in certain trees. Delaying the leaf senescence is a desirable phenomenon in annual crops like rice for better grain filling. Premature senescence of panicle also affects the grain yields in rice. By the use of growth regulators like Kinetin and triactontenol, the panicle senescence can be retarded by maintaining high succinic dehydrogenase activity (SDH) in the panicle components. High rate of N application at booting stage will maintain High SDH activity and retard panicle senescence.

30.5 PHYSIOLOGICAL AND BIOCHEMICAL CHANGES DURING SENESCENCE:

Physiological	**Biochemical**
1. Yellowing of leaves because Of decline in chlorophyll content	1. Decrease in starch synthesis
2. Decrease in photosynthetic rate This may be due to	2. Loss of ATP synthesis
a. Ultra structural changes in chloroplasts	3. Decrease in DNA and RNA Synthesis
b. Decrease in chlorophyll content	
c. Increase in stomata resistance and	
d. Decrease in the activity of Rubisco enzyme	4. Decrease in protein Synthesis. This decline is both due to their accelerated degradation as well as decreased Synthesis
3. Decrease in rate of respiration However in some speciess there is increase in respiration	
4. Increased membrane permeability	
5. Membranus subcelular inclusions are Disrupted	5. Increase in contents of hydrolytic enzymes such as Protease and nuclease.
6. Cells undergo reduction of their structure	6. Decrease in inorganic ions and Various nutrients.

30.6 <u>SIGNIFICANCE OF SENESCENCE</u>: The main purpose of leaf senescence is to recover the nutrients, specially nitrogen and carbon for the growth of younger leaves and other developing organs on the plant. The senescence of leaves in deciduous trees is also a mechanism of avoiding extreme environmental conditions such as severe cold. Of late scientists are trying to develop '**stay green varieties**' which can retard the process of leaf senescence and can maintain green leaves for a longer period.

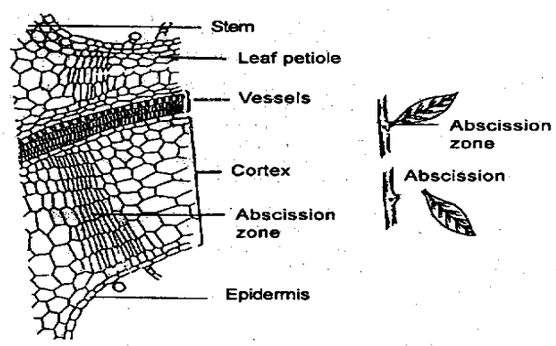
30.7 <u>ABSCISSION AND ITS RELATION TO SENESCENCE:</u>

The term **abscission** is used to describe '**the processes involved in the shedding of plant structure, characterized by the degradation of cell walls at the point of weakening'**. Cells surrounding the fracture line produce and secrete cell wall degrading enzymes which hydrolyze the central region of the wall allowing the cells to separate for fracture to occur. This fracture occurs in "**Separation layer**" which is 1-3 cells wide. Plants do not have the ability to produce separation layer any where. They are genetically limited to specific locations called "**Abscission Zones**" which are 5-50 cells wide.

Cells of abscission zone are somatic and have more persistent meristematic activity. Lignified structural elements like fibers and sclereids are absent in abscission zone and are replaced by collenchymas. Lignified walls are extremely resistant to enzymatic hydrolysis while collenchymas walls are readily degradable.

Abscisic acid was originally isolated as an "**abscission causing factor**". However it is evident that ABA stimulates abscission of organs in only few species and that the primary hormone causing abscission is ethylene. On the other hand ABA is clearly involved in senescence, and through promotion of senescence it might indirectly increase ethylene formation and stimulate abscission. Senescence acts as a signal for inducing abscission and senescence of plant parts usually precedes the abscission.

But there are examples where leaf senescence occurs in the autumn and abscission does not occur until the following spring. Thus, the linkage between senescence and abscission can be broken since, both processes occur independently.



A leaf petiole showing the abscission zone

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Lecture:31

POST HARVEST PHYSIOLOGY

SEED DORMANCY

Seeds harvested at physiological maturity are known to possess maximum viability and vigor. The ability of these seeds to germinate depends on the internal and external environment of the seeds. Any disturbance in external or internal condition leads to failure of germination in seeds.

31.1 Definition: Dormancy is defined as physical or physiological condition of the seed that prevents germination in the presence of otherwise favorable conditions for germination.

Dormancy may occur with in the embryo (Ground nut) or in the seed coat (Sunflower). The period of dormancy varies from a few days to several months depending on the plant species

After-ripening: The period of rest after harvest that is necessary for germination is sometimes referred to as **after – ripening period** and the changes that take place during the rest are described as **after – ripening.**

Quiscence: is the phenomenon in which the seeds fail to germinate for want of a particular environmental factor.

31.2 TYPES OF DORMANCY:

A. Primary dormancy: This is otherwise called as innate dormancy. These seeds enter in to dormancy much before they are harvested i.e when they are still on their mother plant itself.

e.g; 2-3 months in Virginia runners of Ground nut

Up to 40 days after the seed is formed in sunflower.

B. Secondary dormancy: This is called as induced dormancy. These seeds are non dormant when they are harvested from the mother plant. How ever when they are exposed to brief periods of un favorable environmental condition they show dormancy.

e.g: Mustard seed exposed to high concentration of CO_2

Wheat stored in high moisture content in air tight containers at 50^0 C

31.3 ADVANTAGES AND DISADVANTAGES OF DORMANCY

The survival of natural populations depends mainly on their ability to exploit the favorable and avoid the unfavorable weather conditions to which they are cyclically exposed in their natural habitats. The state of dormancy equips organisms

to escape the detrimental effects of adverse natural environments, thereby enhancing their chances of survival.

Plants with a long history of domestication generally show much less seed dormancy than wild or recently domesticated species. Thus crop domestication has promoted rapid seed germinability. This may often result in **premature germination** of seeds within ears/pods (**vivipary**) when the crops are exposed to a wet weather favorable for germination just before harvest. In such cases, a pre-harvest rain leads to deterioration in the quality of crop produce, e.g., in wheat, it reduces seed quality and vigor, milling and baking quality, and even grain yield. Therefore, a certain degree of seed dormancy is often deliberately selected for in order to prevent pre-harvest sprouting in cereals. A brief period of dormancy provides adequate time to farmers to harvest, thresh and store the seeds, thereby avoiding considerable losses.

Disadvantage: The seeds with dormancy cannot be used immediately after harvest for seed purpose.

31.3 CAUSES FOR DORMANCY:

31.3.1 Seed coat factors

(a) Seed coat **impermeable to water**: Common in seeds of Leguminaceae, Malvaceae, convolvulaceae and chenopodiaceae. Seed coat with thick waxy cuticle, lignin and suberin barriers makes seeds impermeable to water.

(b) Seed coat **impermeable to oxygen**: Any disturbance to the entry of oxygen and exit of CO_2 decreases respiration and there by remains in dormant condition.

(c) **Mechanically resistant** seed coat: Hard seed coats of nuts make it difficult for embryo to germinate and break the seed coat. High salt concentration in water also aids to cause mechanical resistance to germination.

31.3.2 Embryo Factors

(a) **Immature/redimentary embryo** : found in *Ranunculus plantago* . These embryos require after ripening though their growth is completed morphologically. This is also called as *physiological dormancy*.

31.3.3 Inhibitory Factors

The inhibitors (e.g : coumarin, caffeic acid , ferulic acid and ABA) may present in the embryo, endosperm or seed coat or pericarp.These inhibitors deactivate enzymes like Amylase, protease and phytase. This will limit the supply of simple substances like sugars, fatty acids and P necessary for

germination. If the ratio of these inhibitors is higher than the that of endogenous hormones especially GA the seeds remain dormant till a balance between them is reached in favor of growth promoters.

31.4 REMEDIAL MESURES / METHODS TO BREAK DORMANCY:

A number of methods of overcoming dormancy have been developed; these methods are either aim at breaking/softening of seed coats or at promoting seed germination through stimulation of embryo. The various treatments for overcoming dormancy may be divided into the following three groups: (I) seed coat treatments, (II) Embryo treatments and (III miscellaneous approaches.

31.4.1 Seed Coat Treatments:

These treatments are either physical or chemical in nature, and aim at making hard seed coats permeable to water and / or gases by either cracking or softening them; the process is usually referred to as **scarification.**

a) Mechanical scarification:

- Rotating the seeds in machines having drums with abrasive surfaces.

- rubbing the seed against abrasive surfaces manually . Eg. Coriander

b) **Chemical scarification:** This is achieved by the use of sulphuric acid, Hcl, NaOH, alcohol, acetone, oxidizing agents etc.

-Treating in 3% nitric acid solution for 6-8 hrs can relieve the seed dormancy in Rice.

Among the several methods available the most suitable method to break seed dormancy at farmers level is nitric acid treatment. - Soaking the seed in o.1 N nitric acid i.e., 6.3 ml per lit. of water for 12 to 24 hours effectively breaks the seed dormancy in less or moderately dormant varieties. However, the varieties like **MTU-1001 (VIJETHA)** which is having 8 weeks and above dormancy duration should be treated with higher nitric acid concentration i.e., 10ml per lit. of water. The seeds can be utilized for sowing immediately after the treatment or they can be dried thoroughly and can be utilized later for sowing.

Scarifications must be done with caution and care; otherwise it may damage the seeds. It may be done in one of the following ways.

- ✓ Scarification may be achieved by rubbing the seeds on a sand paper manually or by using a mechanical scarifier; care should be taken to avoid damage to the embryonic axis. This treatment is effective in species like ‘subabool’, green gram etc.

- ✓ The seed coat may be pierced by a needle or a small incision may be made in it at the abaxial end of the seed, e.g., in bitter gourd (*Memordica charantia*).
- ✓ In some cases, the seed coat may be completely removed by breaking e.g., in rubber (Hevea *Spp*). In this technique, each seed has to be handled individually, which makes the treatment slow, time taking and tedious.
- ✓ The seed may be soaked in a concentrated or dilute solution of sulphuric acid for 1 to 60 minutes, followed by through washing with tap water to remove all traces of acid e.g., in cotton (Gossypium *spp*)

- ✓ In some species e.g. lentil, Bengal gram etc. soaking the seeds in hot water (80^0C) for 1-5 minutes effectively softens their seed coats. But seeds of some species may be highly sensitive to this treatment e.g. a treatment of more than 1 minute reduces the germinability of Bengal gram seeds

Mechanical scarification, especially, manual scarification, is the most commonly used technique and is relatively safer but, quite often tedious.

31.4.2 Embryo treatments

When dormancy is due to factors located within the embryo such treatments have to be applied that are capable of inducing the embryo to resume growth. Some of the common treatments are briefly described below

A. Stratification : stratification is the incubation of seeds at a suitable low temperature (usually,0-5^{o}C) over a moist substratum before transferring them to a temperature optimum for germination; it is a common embryo treatment designed to overcome dormancy. It is commonly used in crops like cherry (*Prunus cerasus*), mustard (*Brassica campestris*)species of family Rosaceae (2-6 months at 5-10^{o}C)etc.

B. High temperature treatment: In some plants incubation at 40-50^0C for few hours to 1-5 days may be effective in overcoming dormancy. Care should be taken that the moisture content of seeds should be less than 15% For example rice (*Oryza sativa*) seeds having less than 15% moisture are incubated at 40-50^0C for 4-5 days for overcoming dormancy.

C. Chemical / Hormonal treatments: The growth regulators most commonly used for this purpose are **GA**, (100ppm is the most commonly used concentration) and **kinetin** (concentration range, usually 10-15ppm). Benzyl adenine at 2 ppm and ethrel at 250ppm are effective in breaking seed dormancy of sunflower. Ethrel 75ppm and GA at 60 – 75 ppm are effective in controlling seed dormancy in groundnut.

Other widely used chemicals are **potassium nitrate** (0.2%) and thiourea (0.5 to 3%) Potassium nitrate breaks the dormancy of seeds requiring light and allows them to germinate in dark e.g. in case of oats, barley

tomato, etc. **Thiourea** breaks the dormancy of seeds requiring light and / or chilling e.g.in lettuce, Gladiolus etc.

31.4.3 Misscellaneous Treatments

Exposing the seeds of many species to red or white Light leads to a termination of dormancy For example lettuce seeds exposed to red light at 660nm or to white light are induced to germinate. Generally seeds are placed initially in red light, and they are subsequently transferred to dark or white light for germination.

31.5 OPTIMUM SEED STORAGE CONDITIONS

Seeds that can be stored in a state of low moisture content are called "**Orthodox**" seeds (Rice, Mung, sorghum, cotton etc.)The seeds that can be stored in a state of high moisture content are called as **recalcitrant seeds (**Cacao, Rubber,Tea etc.**)**. Their viability depends on storage conditions and follows some general rules: (Harrington thumb rules) (ISTA rules) (International Seed Testing Association).

a) For each 1% decrease in seed moisture content, the storage life of the seeds is doubled.
b) For each 10^0F (5.6^0C) decrease in seed storage temperature, the storage life of seed is doubled.
c) The arithmetic sum of storage temperature in degrees F and the percent relative humidity should not exceed 100, with no more than half the sum contributed by the temperature.

These "rules of thumb" clearly indicate that temperature and moisture content of the seed are major factors in determining the viability of seed.

31.6 FACTORS AFFECTING SEED VIABILITY DURING STORAGE:

31.6.1. Seed Moisture:

a) > 30% : Non dormant seeds may germinate
b) 18-30% : Rapid deterioration by microorganisms
c) 8-20% : Seeds respire rapidly and in poor ventilation, the generated heat will kill them.
d) <8-9% : There is little or no insect activity
e) <4-5% : Immune from attack by insects and storage fungi, but they may deteriorate faster than those maintained at a slightly higher moisture content.

31.6.2 Temperature:

Cold storage of seeds at 0-5^{0}C is generally desirable if they are dried to safe moisture limits and sealed in moisture proof containers.

31.6.3 Relative humidity

The activities of seed storage fungi are more influenced by the RH of the inter seed atmosphere than by the moisture content of seeds. Oil seeds may differ in their moisture content from starchy seeds even though both are at same atmospheric RH. At 45% RH starchy seed have 11% moisture and oil seeds have 4-6% moisture.

31.6.4 Cultivar and harvest variability

Different cultivars and harvests of a particular species may show different viability characteristics under the same storage conditions.

31.6.5 Pre and post harvest conditions

Environmental variation during seed development usually has little effect on the viability of seeds, unless the ripening process is interrupted by premature harvesting. Weathering of matured seeds in field particularly in excess moisture or freezing temperatures, results in inferior storage potential. Mechanical damage during harvesting severely reduces the viability of some seeds.

31.6.6 Oxygen pressure during storage:

If seeds are not maintained in hermetic storage at low moisture contents, even under constant temperature and moisture, the gaseous environment may change as a result of respiratory activity of the seeds and associated micro flora.

31.6.7 Fluctuating storage conditions:

Onion and Dandelion seeds stored under conditions of alternating high and low RH lose viability proportionately to the length of time that the seeds are subjected to the high RH.

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Lecture:32

POST HARVEST PHYSIOLOGY

FRUIT RIPENING

32.1 Definition: Ripening may be defined as those nonreversible, diverse, physical, chemical and qualitative changes that render the fruit attractive for consumption at the transition phase following maturation.

32.2 Metabolic changes during fruit ripening :

During the post harvest handling and storage period, and attempt is generally made to maximize control over texutural changes to prevent, synchronize or accelerate the process.

32.2.1 Seed maturation — Quality attributes

32.2.2 Changes in pigmentation — Color

a) Degradation of chlorophyll
b) Unmasking of existing pigments
c) Synthesis of carotenoids
d) Synthesis of anthocyanins

fruit ripening is usually associated with changes in the color of the fruit which is due to changes in the pigment composition of chlorophyll, carotenoids and anthocyanins. In the ripening fruit, there is a fast disappearance of chlorophyll accompanied by accumulation of red and yellow carotenoid pigments in the chloroplast. As the tomato fruit matures, the predominant carotenoid that is synthesized is **carotene.** Some fruits like grapes, pomegranate produces anthocyanins when mature. In tomato another pigment accumulates during ripening is **Lycopene**.

32.2.3 Softening — Texture

a) Changes in pectin composition
b) Possible alterations in other cell wall components
c) Hydrolysis of storage materials

Softening may be a detrimental quality in some fruits like cucumber, squashes which are consumed in unripe state. In others, it is an essential component in the development of optimum quality. With the progress of ripening the fruit softens. The softening is due to enzymatic hydrolysis of polysaccharides. The cell wall is made up of cellulose, hemi cellulose, calcium pectate, polyuronides, and glycol protein. The important cell wall hydrolyzing enzymes like pectin methyl esterase (PME), poly galacturenase (PG) and cellulose increases during ripening and the dissolution of middle lamella is observed. This is accompanied by increase in the enzyme PME.

Another enzyme poly galacturonase (PG) which is not present in green tomato appears during ripening. This enzyme hydrolyses oligo galacturonoids.

32.2.4 Change in carbohydrate composition.
a) Starch conversion to sugar
b) Sugar inter conversions
32.2.5 Production of aromatic volatiles
32.2.6 Changes in organic acids

} Flavor

During ripening, starch hydrolysis occurs, and sugar accumulates. For example starch content of banana decreases from the initial 21% to about 1% in ripened fruit. This is accompanied by accumulation of sugars mainly sucrose to the extent of up to 20% by fresh weight. The taste of the fruit depends upon the sugar-acid ratio and also on the absolute level of sugar and acid contents. The PH of all the fruits is in the acidic range.

32.2.7. Fruit abscission occurs.
32.2.8 Changes in respiration rate (respiration rate increases).
32.2.9 Increased permeability of tissue.
32.2.10. Quantitative and qualitative changes in protein occur.
32.2.11 Development of surface waxes occurs.

32.3 FACTORS INFLUENCING FRUIT RIPENING

A)Temperature affects the rate of synthesis of specific pigments and their final concentration in the fruit. The optimum and maximum temperature for synthesis of a specific pigment varies between species. For eg. Lycopene synthesis in tomato is inhibited above 30^0C whereas in watermelon synthesis is not prevented until the fruit temperature rises above 37^0C.

B)Oxygen is essential for carotenoid synthesis and increasing the oxygen concentration enhances the synthesis of this pigments.

32.4 Climacteric and non- climacteric Fruits: Fruits of different species vary in their ability to ripe, when detached from the parent plant. Many fruits must be harvested only when fully ripe eg. **Grape, cherry, lemon** etc. they are not capable of ripening after detachment and are called **non climacteric fruits**. Other fruits **like apple, banana, tomato** can be harvested unripe but after fully mature stage. They can undergo normal ripening even though detached from the parent plant and are called **climacteric fruits**. These fruits often, undergo hydrolytic conversions in storage materials and synthesize the pigments and flavors associated with a ripe fruit.

32.5 HORMONAL REGULATION OF RIPENING

32.5.1 Ripening Induction

Application of ethephon promotes degreening and early ripening in grape, tomato, coffee, peach, pear, plum and citrus. Smoking is commercially employed to hasten degreening and ripening of banana and mango. Calcium carbide release acetylene, on hydrolysis of which hasten ripening process. ABA (1 ppm) Thirourea (20%), CCC (4000ppm) (2 chloro ethyl – trimethyl ammonium chloride), Etherel (200-300 ppm) sprays one week before harvest hastens ripening.

32.5.2 Delay of Ripening

The self life of fruits like apple, banana and other can be improved by storing the fruit in low oxygen tension (2-3%) or by absorbing ethylene with a suitable absorbent like alumina or silica gel impregnated with potassium permanganate. Another commercial practice is to preserve the fruits in cold storage. Maleic Hydrazide, GA(10^{-6}M), IAA (10^{-6}M), Kinetin (10^{-5}M), sprays one to two weeks before harvesting and post harvest dip of cycocel, Alar, GA (150 ppm), vit K_3 (menadione Sodium bisulfite), $KMnO_4$, $CaCl_2$, waxol delays ripening.

32.5.3 Sugarcane Ripening

Glyphosine, glyphosate and ethephon hasten ripening of sugarcane. Other compounds like chlormequat, mefluidide, Polaris and ripenthol also used for sugarcane ripening.

32.5.4 USE OF HORMONES TO IMPROVE SHELFLIFE OF CUT FLOWERS

The use of preservative solutions to promote the quality and longevity of cut flowers is known for many years. Flower preservatives are composed mainly of sugars and germicides and some times include mineral solutes, organic acids, salts, antioxidants and ethylene inhibitors. Use of hormones in preservation solution is relatively limited. However cytokinins are widely used.

Sl. No.	Type of compound	Crop plants	Optimum conc. And purpose	Nature of action
1	Cytokinins (kinetin, BA, IPA, PBA)	Carnations, rose, iris, tulip, gerbera, anthurium	• 100 ppm for pulsing • 10-100 ppm for bud opening & holding solution	• Ethylene production is reduced. • Burst in ethylene production is delayed. • Improved water uptake and maintained turgidity.

2	Auxin (2-4D)	Carnation	• 500 ppm for holding solution	• Inhibits ethylene production • Retards petal senescence
3	GA3	Carnation gladiolus	• 200 ppm holding solution, • 20-35 ppm for bud opening	• Delays senescence
4	ABA		• 1 ppm in continouos holding solution • 10 ppm for 1 day	• Delays wilting by stomatal closure
5	Growth retardants B-Nine	Snap dragon carnation roses	• Snapdragon 10-50 ppm • Carnation & roses – 500 ppm for pulsing & 125 ppm for holding solu	• Delays senescence
	Chlormequat	Gladiolus, tulip & carnation	• 20-50 ppm for bud opening • 10 ppm for holding solution	• Delays senescence
6	Inhibitors Eg. MH	Snap dragon, lupine	• 0.5-1% for 30 min for pulsing • 250-500 ppm for holding solution	• Reduces respiration of flowers • Slows down metabolism, ageing & development

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