**Dr. Rima Kumari: Date: 06/07/2020**

Online class and e- content for M.Sc. II semester students

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| Date and Time | Online class medium  | E. content topic |
| 06/07/20201:30 p.m to 2.30 p.m | Via Google meetLink: Meeting URL: <https://meet.google.com/fhr-scis-wzj> | Plant Growth and Development,Plant Growth Hormones: auxin, gibberellic acid, cytokinin, |

**M.Sc. Semester II**

**MBOTCC-7 Physiology and Biochemistry**

**Unit 3**

Plant Growth and Development, Plant Growth regulators

**Definition of Growth**

Growth is “an irreversible process of increase in size of cell, tissue or an organ or an individual plant. Growth is the most fundamental and basic characteristics of all living beings and is growth occur as a result of several metabolic processes. These metabolic processes may be catabolic or anabolic. In successive growth stages in plants, seed germinates, develops into seedling and later it takes the shape of an adult plant.

## **Characteristics of Growth**

* **Plant Growth is generally Indeterminate –** **Plants** possess the ability of growth throughout their life. This is due to the presence of meristems at certain locations in their body and these meristems have the ability to divide and self –perpetuate.
* **Growth is Measurable –** At cellular level, Growth is the consequence of increase in protoplasm but this increase is difficult to measure. Growth, in plants, is measured via different methods like increase in height, fresh weight., dry weight, volume, cell number,

**The Growth of Plants has three phases:**

* **Formative Phase –** Cell division is the basic event in the growth of plant. All cells are the result of division of pre-existing cells. Mitosis is the type of cell division that happens during growth. This division is carried out in two steps – Division of Nucleus (Karyokinesis) and division of cytoplasm (Cytokinesis). In case of higher plants, an increase of cells is carried out in meristematic region, whereby some daughter cells retain the meristematic activity while some enter in the next phase of growth, i.e. the phase of cell enlargement.
* **Cell Enlargement and Cell Differentiation** – At this stage, the size of tissues and organs is increased and this enlargement occurs by forming Protoplasm, Hydration (absorbing water), developing vacuoles and then adding new cell wall to make it permanent and thicker.
* **Cell Maturation** – At this stage, the enlarged cells acquire specific size and forms as per their location and function. Thus, several cells are differentiated from simple and complex tissues which perform different functions.

**Experiment to Study Phases of Growth**

In order to study the phases of Growth, germinate few seeds of peas in moist cotton bed in petri plate. Select the couple of seedlings with 2 – 3 cm of length, wash them and blot the surface water. Then, mark the radicles from tip to base with 10 – 15 point at interval of 2 mm via water proof ink. After drying of ink, place those seedlings on moist blotting paper and allow them to grow for 1 – 2 days. Finally measure the intervals between the marks and we can clearly observe the different phases of growth.



Ist stage in seed, II nd stage seed germination and the marked radicle of seedling at the beginning of experiment and next condition is after 48 hours. We can clearly identify hypoctyl region. Zone of hypoctyl at maturity form shoot region and radicle form roots.

Different zones of cell growth i.e., zone of cell formation, cell elongation, cell differentiation and zone of matured cells is described in picture given below



Types of Growth

**Primary and Secondary Growth:**

The mitotic divisions of meristematic cells (undifferentiated cell capable of cell division) present at the root and shoot apex increases the length of the plant body. This is referred as Primary Growth. Secondary meristem results in an increase in diameter of the body of plant.i.e., stem girth

**Unlimited (Indeterminate) Growth:** This is the stage, when root and shoot of plant continuously grow from germination stage to death and throughout the entire lifespan.

**Limited Growth:** This is the stage, when fruits, leaves and flowers stop growing after attaining certain size. It is also called determinate type of Growth.

**Vegetative Growth:** The Growth of Plant before flowering in called Vegetative Growth. This Growth includes producing of stems, leaves and branches.

**Reproductive Growth:** At this stage, plants start flowering, which is the reproductive part of the plant.

Factors Affecting Plant Growth

External Factors: Oxygen, Water and Nutrients, Temperature and Light.

**Temperature:**

Temperature plays important role in the growth of plants. The minimum, optimum and maximum temperature varies and from species to species. As the temperature increases above minimum, growth is accelerated until the optimum temperature is attained, when the growth gets slower and is completely retarded. Effect of duration for which a plant is exposed to certain temperature also varies amongst different species. For Example: The plant shows good growth when it is exposed to 86°F for a short duration and the same temperature has negative impact if maintained for longer duration.

**Light**

Light also affect the growth and development of plant. Several factors of light like light intensity, duration of light and quality of light influences several physiological processes like movement of stomata, chlorophyll synthesis, temperature of aerial organs, formation of anthocyanin, absorption of minerals streaming of protoplasm and rate of transpiration. Intensity of light also influences plant growth and the variation in intensity has significant impact on growth pattern. Most ornamental plants and crops, such as Peas, Corn, Tobacco and Peas makes stocky and vigorous growth will full sun and thus, is also called “Sun Plant.”

Difference in wave length of light also effects the growth of plant. Several experiments have proved that plants that has full spectrum of visible light shows proper development and increase in dry weight. Plants grown in violet and blue light tend to dwarf, while plants in red light are taller and spindly.

Duration of light also affects the plant growth as it affects the rate of photosynthesis. For instance, during winters when days are short, the growth is very slow, while, it increases during summers when the days are longer.

**Oxygen:**

The plants with lesser availability of oxygen show retarded growth while it is vice versa in the presence of ample of oxygen. It is important to note that plants in flooded areas, results in deficiency of soil aeration which on the other hand, results in poor plant growth.

**Water**

Water is very important for plants and inadequate water results in poor growth. Plants grow well only in the presence of optimum water. Plants respond to deficiency of moisture as well. For instance, peppers, spinach and radishes wilt and cease to grow when the percentage of water in soil is lower.

**Soil nutrients**

Soil nutrients, their quantity and nature also affect the growth of plant. For Luxuriant Growth, it is important to have adequate amount of nutrients.

## **Important terminologies:**

## **Differentiation, Dedifferentiation and Redifferentiation**

**Differentiation:** The cells derived from root apical and shoot – apical meristems and cambium differentiate and mature to perform specific function and this act leading to maturation is termed as differentiation.” During this process, **several** structural changes are carried out in cells and protoplasm. For instance, In order to form a tracheary element, cells would lose protoplasm and develop elastic, strong and lignocellulosic secondary cell walls in order to transport water even in extreme tensions.

**Dedifferentiation:** An undividable differentiated cell sometimes regains the power of division. This process is called dedifferentiation. Dedifferentiation is a common process in plants during secondary growth and in wound healing mechanisms. A dedifferentiated cell can divide and produce new cells.. **For Example:**Formation of Meristems – cork cambium and interfascicular cambium form fully differentiated parenchyma cells and in such condition, tissues and meristems are able to divide and produce cells even after losing the capacity to divide.

**Redifferentiation:** produced new cells of dedifferentiated cell again loose the power of division and become a part of permanent tissue. This process is called “redifferentiation’. Tumour cells form good example for redifferentiated cells.

**Growth pattern in plants:**

The increased growth per unit time is termed as **Growth Rate**. An organism can produce cells in several ways and display **Geometric**as well as **Arithmetic Growth.** Following diagram shows both types of growth in plants:



The following diagram displays the various stages of embryo development showing both **Geometric**and **Arithmetic Phases**. Here dark blue blocks **represent**the cells capable of division while light blue blocks represents the cells that have lost the capacity to divide:

Thus, in **Arithmetic Growth**, only one daughter cell continues to divide while other differentiates and matures. The following graph represents the length of an organ against time, whereby a linear curve is obtained. We can clearly observe the constant linear growth against time t.

**Geometrical Growth:**  In majority of cases, Initial Growth is slow and is referred as lag phase. Then, it increases rapidly at an exponential rate referred as log phase or exponential phase. The growth of plant slows down in cases of limited nutrient supply and results in stationery phase. When we plot the growth against time, it results in **S-Curve**or **Sigmoid Curve**. Following graph represents an idealized sigmoid growth curve typical of cells in culture and many higher plants and plant organs.

 It is an ‘S’ shaped curve obtained when we plot growth against time (Fig. 15.2). It is also called ‘sigmoid ‘curve. This curve mainly shows four phases of growth- 1.initial slow growth (Lag phase), 2. the rapid period of growth (log phase/grand period of growth/exponential phase) where maximum growth is seen in a short period and 3. The diminishing phase where growth will be slow and 4. Stationary / steady phase where finally growth stops.



Plant embryonic cell showing arithmetic and Geometric growth is presented below



**Plant growth regulators:**

Light, water, oxygen and nutrition is obligate requirement for plants to grow and develop into fully matured plants. Along with these environmental factors, plants also produce intracellular chemicals which help in their growth and development. These factors are called plant growth regulators. They are intrinsic factors. So, Plant Growth Regulators are simple chemicals produced naturally by plants to regulate their growth and development.

**Characteristics**

These chemicals are having diverse chemical composition. They are also referred to as plant growth substances, phytohormones or plant hormones. Based on their chemical nature and mode of actions important plant growth regulators are ethylene (gaseous form), auxin, gibberellic acid, cytokinin, abscisic acid. .

Based on their action, they are broadly classified as follows:

**Plant Growth Promoters** – They promote cell division, cell enlargement, flowering, fruiting and seed formation. Examples are auxins, gibberellins and cytokinins.

**Plant Growth Inhibitors** – These chemicals inhibit plant growth and promote dormancy (temporary inactive phase) and abscission in plants (natural detachment/ falling off of dead leaves and riped fruit. An example is an abscisic acid.

Ethylene helps in fruit ripening. It can be a promoter or an inhibitor, but is largely a Plant Growth Inhibitor.

**Auxins**

Natural auxins produced by plants are Indole-3-acetic acid (IAA) and Indole butyric acid (IBA), phenylacetic acid (PAA), indole-3-propionic acid (IPA). Natural auxins are found in growing stems and roots coleoptile from where they migrate to their site of action. Naphthalene acetic acid (NAA) and 2, 4-dichlorophenoxyacetic (2, 4-D) are examples of synthetic auxins.

**Discovery**

Auxins were the first growth hormone to be discovered. They were discovered by observations of Charles Darwin and his son, Francis Darwin. The Darwins observed that the apical coleoptile (protective sheath) in canary grass grows and bends towards the source of light. This phenomenon is ‘phototropism’. In addition, their experiments showed that the coleoptile tip was the site responsible for the bending. Finally, this led to the isolation of the first auxin by **F.W. Went** from the coleoptile tip of oat seedlings. He work on oat (*Avena sativa*), he reported that If the growing tip of oat coleoptile is removed, the remaining portion of coleoptile will show a marked decrease in growth which will ultimately stop. That showed that chemical present in growing tip of oat coleoptile help in plant elongation. This substance is t/a IAA (indole-3-acetic acid), natural auxin.. Went (1928) performed Avena-curvature test.



**Avena Curvature Test:**

[Went](https://en.wikipedia.org/wiki/Frits_Warmolt_Went) showed that a growth promoting chemical diffuses from coleoptile tips, and causes a coleoptile to grow towards the light. Went cut the tips of the coleoptiles and placed them in the dark, putting a few tips on agar blocks then he predicted that agar would absorb the growth-promoting chemical. On control coleoptiles, he placed a block that lacked the chemical. On others, he placed blocks containing the chemical, either centered on top of the coleoptile to distribute the chemical evenly or offset to increase the concentration on one side. When the growth-promoting chemical was distributed evenly the coleoptile grew straight. If the chemical was distributed unevenly, the coleoptile curved away from the side with the cube, as if growing towards the light, even though it was grown in the dark. Went later proposed that the messenger substance is a growth-promoting hormone, which he named auxin, that becomes asymmetrically distributed in the bending region. Went concluded that auxin is at a higher concentration on the shaded side, promoting cell elongation, which results in coleoptiles bending towards the light



**Avena curvature test**

**Physiological role of Auxin**

1) Apical dominance: The growing apical bud inhibits the growth of the lateral buds

2) Root initiation: Auxin helps to initiate rooting in stem cuttings

3) Flowering: Auxins promotes flowering in plants

4) Abscission: Auxins promote the abscission (natural detachment) of older leaves and fruits, but prevent dropping of fruits and leaves too early.

5) Control xylem differentiation

6) Cell division: Auxins help in cell division

7) Induce parthenocarpy i.e. the production of fruit without prior fertilization.

8) 2, 4-D (synthetic auxin) is widely used as a herbicide to kill dicotyledonous weeds.

**Apical Dominance (definition):** The growing apical bud in higher plants due to auxin at apical region inhibits the growth of the lateral buds. This phenomenon is ‘Apical Dominance’. Removal of the apical bud allows the lateral buds to grow. This technique is commonly used in tea plantations and hedge-making.



**Apical dominance**

**Natural auxins:**

Indole 3-acetic acid (IAA) are naturally occurring auxins in plants and therefore; regarded as phytohormones. is the best known and universal auxin. It is found in all plants and fungi. Besides IAA, indole-3-acetaldehyde, indole -3-spyruvic acid, indole ethanol, 4-chloro-idole aerie acid (4-chloro-­IAA) etc., are some other natural auxins.

The first naturally occurring auxin was isolated by Kogl and Haagen-Smit (1931) from human urine.

**Physiological Effects and Applications of Auxin**

1. **Apical Dominance** (Characteristic function of auxin): The phenomenon in which apical bud dominates over the growth of lateral buds is called Apical Dominance. Prunning in gardens promotes densing of hedge.
2. **Cell Division & Cell Enlargement/Callus formation** Auxin is important in Tissue culture & Grafting. It stimulates division of intrafascicular cambium. Also in healing of wounds.
3. **Shortening of Internodes:** a-NAA induces the formation of dwarf shoot or spurs in apple, pear etc., thus number of fruits increases.
4. **Prevention of lodging:** Auxin spray prevents lodging of crops, immature leaves & fruits.
5. **Root initiation:** Rooting on stem cuttings is promoted by IEA & NAA (Root growth inhibited by auxin).
6. **Potato dormancy**: MH (Maleic-Hvdrazide). a-­NAA., induces dormancy of lateral buds in potato tubers & potato can stored for long duration.
7. **Prevention of Abscission:** IAA, NAA prevents premature abscission of plant organs.
8. **Flower initiation:** Auxin is inhibitor of flowering but it promotes uniform flowering in Pine apple & Litchi plants.
9. **Parthenocarpy**: Seed less fruits can produced by spray of IAA. (By Gusteffson)
10. **Selective weed killer:** Dicot broad leave weeds can be eradicated by 2, 4-D & 2, 4, 5-T.
11. **Agent orange** is used in biowar. It was used by USA against Vietnam (1966-60).
12. **Femaleness:** Feminising effect in some plants.
13. Flower & Fruit thinning: Certain trees like mango form less number of fruits in alternate years. But auxins can produced normal fruit crops every year. This is known as fruit thinning.
14. When Antiauxin (TIBA-Tri-Iodo-Benzoic acid) are sprayed on mature cotton field then cotton balls can picked easily.

**Bio-Assay test:**

Bioassay means the testing of substance for it's activity in causing a growth response in a living plant or it's parts.

(i) **Avena curvature test**: Avena curvature test carried out by F.W. Went (1928), demonstrated the effect of auxins on plant growth by performing some experiments with the oat (Avena sativa) coleoptile.

(ii) **Root growth inhibition test:** are bioassays for examining auxin activity.

**Gibberellic Acid/ Gibberline**

First of all Japanese farmers observed peculiar symptoms in rice seedlings & called the bakanae disease (Foolish seedling disease). In this disease, rice plants become thin, tall & pale due to infection of Gibberella (Ascomycetes) or Fusarium (Duteromycetes) confirmed by **Kurosawa & Swada**.

**Yabuta and Sumiki 1938** were first to extract a crystalline substance from the Gibberella fungus, which they named as Gibberellin.

Gibberellin is acidic, 100 type of Gibberellins (GA1, GA2, GA3, ……… GA100) are known. GA3 [C19H26O6] is representative of all Gibberellins. First discovered Gibberellins from higher plants was GA1 (GA1 & GA20 are common GA's of higher plants)

GA found in all group of plants (algae to angiosperms) but as a flowering hormone acts only in angiosperms., Biosynthesis of gibbereilin takes places by Mevalonic acid pathway (Kaurene ® GA)

**Physiology Effects and Applications**

**Stem/internode elongation**: It is the characteristic function of gibberellins. GA induces internode elongation, leaf expansion & used in sugarcane cultivation.(Gibberellins induce stem elongation in Rossete plants (Cabbage). This phenomenon known as Bolting effect. (Elimination of rosset habit in some plants by gibberellins action is called bolting)

**Elongation of genetic dwarf plants** When gibberellin are applied to dwarf *Maize, Pisum &. Vicia faba*, then they become tall. Extreme dwarfism in rosette plant/ dwarfism can be eliminate by GA.

**Flowering in Long Day Plant** in short right duration

**Parthenocarpy:** Like Auxin, exogenous use of GA also induces the formation of seedless fruits.

**Substitution of cold treatment or Vernalisation**

**GA induce flowering** in biannual plants even in first year form

**Breaking of dormancy**: GA breaks the dormancy of seeds, buds and tubers.

**Seed germination**: Gibberellins induce germination via activating the synthesis of hydrolysing enzymes like oamylse, Lipases & Proteases, induce pollen tube germination process.

**Sex expression:** GA induce maleness in Cucumis, Cannabis.

**Germination of Photoblastic seeds**: Gibberellin treated light sensitive seeds can germinates in dark. Ex. Lettuce, Tobacco.

**Fruit & Flower enlargement:** Size of grape fruits & bunch & Geranium flowers can be increased by GA. Pomalin & GA (GA4 & GA7) + CK(6- Benzyladenine) - acts as apple enlarger.

**Bio assay:**

1*. a-amylase activity test in Barley endosperm:*

Endosperms are detached from embryos, sterilized and allow to remain in 1ml of test solution far 1-2 days. There is build up of reducing sugars which is proportional to GA concentrations. Reducing sugars do not occur in endosperms kept as control.

*2. Elongation of Dwarf Pea & Maize test:*

Seeds of dwarf pea are allowed to germinate till the just emergence of plumule. GA solution is applied to some seedlings, others are kept as control. After 5 days, epicotyl length is measured. Increase in length of epicotyl over control seedlings' is proportional to GA concentration.

**Cytokinins(CK)**

Cytokinin was discovered by Miller and prof. Skoog) on tobacco pith culture. They added the contents of an old DNA­ bottle (Herring fish sperms DNA) to the culture medium & observed that the-tobacco pith callus could grow for longer period.

Miller isolated an active substance from autoclaved DNA from Herring sperm, stimulated cell division. He named this substance as kinetin

**Cytokinin: important points to remember**

1. Term cytokinin By Letham and Skoog.
2. The first natural cytokinin was identified & crystallized from immature corn grains by Letham & named as Zeatin.
3. The most common cytokinin in plants are Zeatin and isopantanyl adenine.
4. Cytokinin is a derivative of Adenine base.
5. Root tips are major site of synthesis of CK (by Mevalonic acid pathway).
6. Movement of cytokinin is polar & Basipetal.
7. Coconut milk factor also performed activity like cytokinin, thus used in tissue culture.
8. Zachau obtained cytoknins from serine-t-RNA of yeast.
9. BAP (Benzylamino purine), Diphenylurea and Thidiazuron are synthetic cytokinins.

**Physiological Effects and Applications**

1. **Cell Division: & Cell Enlargement:** One of the most important characteristic function of cytokinin on plants is induction of cell division. In tissue culture also.
2. **Induction of secondary growth**: Formation of inter fascicular cambium and induce secondary growth.
3. **Morphogenesis:** Morphogenetic changes induce by CK in presence of IAA.

High CK + low auxin - Shoot formation

High auxin + low CK - Root formation

1. **Promotion of lateral growth**: Promotes growth of lateral buds by counteracting/ masking apical dominance:
2. **Breaking the dormancy of seeds**: Like GA the dormancy of certain seeds can be broken by CK.
3. **Delay in senescence (Richmond Lang effect):** The ageing process of leaves usually accompanies with loss of chlorophyll and rapid catabolism. This is called as senescence. Senescence can be postponed by CK (increase short life of plant parts)
4. **Lignin biosynthesis** in some plants
5. **Parthenocarp**y in some fruits
6. **Phloem conduction** : induce acilitation of Nutrients Mobilisation in all parts of plants
7. **Femaleness**: induce femaleness in plants
8. **Flowering in Short Day Plant**: Induce flowering in SDP even in in long days duration)
9. **Induce stomatal opening**

**Bio-assay:**

**1. Tobacoo pith cell division test:** Tobacco pith culture is divided into two weighted lots. One supplied with cytokinin and the other without it. After 3-5 weeks, increase of fresh weight of treated tissue over control is noted. It is a measure of stimulation of cell division and hence cytokinin activity.

2. **Delay in senescance test:** Leaves are cut into equal sized discs with the help of a cutter. They are divided into two lots. One lot is provided with cytokinin. After 48-72 hours, leaf discs are compared for chlorophyll contents. Cytokinin retards chlorophyll degradation. Lower loss of chlorophyll pigmentation under cytokinin treatments results to delay in plant senescence process

**Gibberellins (Physiological Effects in detail): (contd.)**

1. **Substitute for light in Long Day plant**

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

1. **Breaking the dormancy of Buds:**

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

1. Inhibition of Root Growth:

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

1. **Elongation of the Internodes:**

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

1. Bolting and Flowering:

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

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

1. Parthenocarpy:

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

1. Induced Light Inhibited Stem Growth:

It is common observation that the dark grown plants become etiolated and have taller, thinner and pale stems while the light grown plants have shorter, thicker and green stems, and it may be concluded that light has inhibitory effect on stem elongation. Treatment of light grown plants with gibberellin also stimulates the stem growth and they appear to be dark brown. In such cases the protein content of the stem falls while soluble nitrogen content increases prob­ably due to more breakdowns of proteins than their synthesis.

It is considered that the light in some way lowers the level of endogenous gibberellins and inhibits the stem growth.

1. De novo Synthesis of the Enzyme-α-Amylase:

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

**Mode of Action of Gibberellins**

1. **Gibberline stimulate both Cell Division and Cell Elongation:**

Stem elongation in plants as a result of gibberellin treatment involves both cell division and cell elongation. In rosette long day plants, GA treatment causes marked increase in mitosis in the sub-apical regions of apical meristems. Internodes of tall pea plants have more cells than in dwarf ones and their cells are longer in size too. In deep-water rice, stimulation of internodes elongation is partly due to increased cell di­visions in the intercalary meristems and partly due to elongation of cells of the latter who have divided with cell elongation preceding the cell divisions.

**GA Stimulated Cell Divisions are Regulated Between G2 and M Phases of Cell Cycle:**

The mechanism of GA stimulated cell division has been extensively studied in intercalary meristems of young internodes of deep-water rice. In this case, the GA – stimulated cell divisions are believed to be regulated between G2 and M phases of cell cycle. The transitions between different phases of cell cycles are known to be regulated by cyclin- dependent protein kinases (CDKs). GA stimulates cell divisions by increasing the expression of two genes (CDC2) that encode CDKs and M cyclins which are required for entry into mitosis.

1. **Mobilization of Endosperm Food Reserves:**

As mentioned earlier, the GAs cause de novo synthesis of a -amylase in cells of aleurone layer of germinating cereal grains. (The GAs synthesized by coleoptile and scutellum of the embryo are released into the starchy endosperm from where they diffuse into the cells of aleurone layer). GAs also bring about secretion of a -amylase and other hydrolytic enzymes from the cells of aleurone layer into the starchy endosperm where complex carbohydrates are hydrolyzed into simple sugars which are then trans located to growing embryo to provide energy source. Although the mechanism of induction of a-amylase synthesis in cells of aleurone layer by GA and its secretion from cells of aleurone layer into the endosperm, have been extensively studied, but they are yet far from being completely elucidated.

**Plant Responses to Cytokinins (in detail)**

Cytokinins play a prominent role in all the phases of plant development from cell division and enlargement to the formation of flowers and fruits. They increase resistance to aging and to adverse environment.

 **(i) Cell Division:**

For continued in vitro growth and cell division of tissue accompanied by DNA synthesis, cytokinin is necessary along with auxin. While auxin and gibberellin are also able to stimulate DNA synthesis and mitosis, cytokinin alone can stimulate **cytokinesis**. Quite opposite to the pro-motive effect of auxin and gibberellin, cytokinin inhibits elongation of stem sections. Root growth is generally inhibited by cytokinins.

**(ii) Cell Enlargement:**

Cytokinins may stimulate radial growth of stem tissue by swelling rather than by longitudinal extension. Vine well-known leaf enlargement caused by cytokinin is due to an effect on cell enlargement rather than cell division. In fact, cytokinin appears to promote overall enlargement of cells and not simply elongation. Cytokinin effect on cell enlargement may be due to an influence on micro fibril orientation from longitudinal to radial direction.

**(iii) Tissue Differentiation:**

Organs in tissue culture show a spectacular response to cytokinin. With a low cytokinin supply, the tissue remains as an amorphous undifferentiated callus.

Bud formation and shoot initiation depend on higher concentrations of cytokinin by changing cytokinin auxin ratios. An interesting observation on morphogenesis in tobacco callus cultures is that a high cytokinin auxin ratio results in the production of shoots but no roots, but a low ratio leads to an opposite effect producing roots only.

In addition to their role in leaf expansion, cytokinins also play a regulatory role in chloroplast formation. When cytokinin is absent, plastids are formed but remain undifferentiated. Presence of both light and cytokinin is necessary for grana development and conversion of pro-plastids into chloroplasts.

**(iv) Retardation of Senescence:**

The retardation of senescence by cytokinin is a well-known phenomenon. Richmond and Lang first discovered that when leaf discs are kept in water, senescence appears within a few days as evident by the loss of chlorophyll and protein. But when cytokinin is added to the leaf discs, senescence is delayed through the maintenance of chlorophyll and protein.

This senescence-retarding property of cytokinin as mediated through the retention of chlorophyll is known as **Richmond-Lang** effect.

**(v) Mobilization of Nutrients:**

Mothes observed that when a particular area of leaf is treated with cytokinin, that treated area remains green showing delay of senescence, while the untreated area loses its green colour and becomes yellowish showing symptoms of senescence. Here the nutrients are drawn or mobilized from other parts of leaf so that the treated area remains green at the expense of the untreated area.

**(vi) Release of Dormancy of Seeds and Buds:**

Applications of cytokinins can stimulate germination and break dormancy. One of the remarkable characteristics of cytokinins is their ability to modify the effects of other hormones without any marked effects by themselves.

When dormancy is imposed either by high temperature (thermo dormancy) or by an accumulation of inhibitor like ABA (inhibitor dormancy), then GA alone is not capable to overcome dormancy. Addition of cytokinin opposes the action of inhibitor and permits germination.

Thus cytokinin has been documented as a permissive agent in germination by antagonizing the inhibitor action — a case of cytokinin-inhibitor antagonism. In bud growth inhibitor (preventive) and cytokinin (permissive) show opposite effects. Thus inhibitor-induced bud dormancy can be overcome by cytokinin.

**(vii) Masking of Apical Dominance:**

Cytokinin applied on lateral buds is able to mask from the effect of apical dominance whether it is due to the presence of terminal bud or due to applied auxin. This has been interpreted as an increase in IAA transport and mobilization of metabolites from the apical region to the point of application of cytokinin which is supported by the striking influence of cytokinin on phloem transport.

**(viii) Resistance to Adverse Factors:**

Cytokinins increase the resistance of plants to adverse factors such as high and low temperatures and certain disease. The nature of the action of cytokinins in bringing about these effects is still unknown.

Naturally-occurring cytokinins have been implicated in host-parasite relationship. Infection by bacterium *Corynebacterium fascines* which produces cytokinin leads to the fasciation disease symptoms in many plants. Treatment with cytokinin may induce a similar pathogenic condition.

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Cytokinin has a distinct action on the mechanism of stomatal movement. Although the stomatal aperture in the isolated epidermal systems is not much influenced by cytokinins, treatment of whole leaf with cytokinin has been reported to increase the stomatal aperture and thereby transpiration.

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The development of inflorescence is influenced by cytokinin treatment by increasing both the number and size. Cytokinin has been shown to cause a male-flowering plant to produce hermaphrodite flowers. Enhancement of fruit set and fruit size in grape varieties and induction of parthenocarpy in fig have also been reported.

**Mode of Action in Cytokinins:**

(i) Control of Transcription and Translation:

At present, the biochemical basis of cytokinin action is still not completely known. Still there are many evidences which make it clear that cytokinins greatly influence nucleic acid metabolism. Guttman first reported that kinetin treatment of onion roots caused a rapid increase in nuclear RNA and this observation was later confirmed by Jensen et al.

**cytokinins effect on nucleic acid synthesis**:

 Both the chemical constitution of the cytokinins and their effect on nucleic acid synthesis strongly suggest that they exert their biological activity directly in nucleic acid metabolism. Presence of cytokinin activity in specific tRNA species would suggest the influence of cytokinins on specific rather than bulk protein synthesis. Location of the cytokinins next to the anticodon in certain tRNA species with known base sequences suggests that they may function specifically in the translation step of gene- controlled protein biosynthesis.

Since various developmental pathways are influenced by cytokinins, it is pertinent to expect cytokinins to control the synthesis of many proteins either by regulating the transcription of the genes encoding these proteins or by an effect at the post-transcriptional level.

Induce the synthesis of nitrate reductase enzyme:

One well-known protein, the synthesis of which is induced by cytokinin is nitrate reductase enzyme. Nitrate reductase is a cytosolic enzyme, which reduces nitrate (NO3) to nitrite (NO2). Further reduction of nitrite to ammonia (NH+4) is catalysed by the chloroplast enzyme nitrite reductase. It is interesting to note that nitrate metabolism is induced by light, which initiates the expression of both the nitrate reductase gene encoded by nucleus as well as the chloroplastic nitrite reductase gene. Cytokinins can stimulate the synthesis of nitrate reductase in dark-grown leaves, which suggests that the light requirement for the induction of this enzyme can be partly substituted by cytokinins.

It has been further observed that along with the induction of nitrate reductase by cytokinin, there is a corresponding increase in nitrate reductase mRNA. Since cytokinin-stimulated nitrate reductase can be blocked by inhibitors of both gene transcription and protein synthesis, it may be concluded that cytokinin possibly exerts its effect both at the levels of transcription and translation.

(ii) Respiratory Enzymes and Metabolism:

In cultured tissue, respiratory activity can be increased by the addition of cytokinins. It has been shown that cytokinin stimulation of respiration involves a suppression of glycolytic enzymes and a shift to the hexose monophosphate shunt. In intact systems, high doses of cytokinins cause inhibition of respiration and delay of senescence can be correlated with decrease in respiration. Cytokinins have been found to influence the activity of a number of specific enzymes. Cytokinin induces the formation of tyramine methyltransferase which catalyses thiamine synthesis.

 **(iii) The Richmond-Lang Effect:**

The Richmond-Lang effect suggested that cytokinins play an active role in senescence retardation in detached leaves. The mechanism is based on the postponement of the disappearance of chlorophyll and the degradation of proteins through the activity of the corresponding hydrolases which normally accompany the senescence process.

Mothes suggested that the primary function of cytokinin in this respect is to increase the amino acid accumulation and to increase or retain the protein content.

**(vi) Cytokinin and Auxin Regulate Plant Cell Cycle:**

Both auxin and cytokinins are involved in the regulation of cell cycle by controlling the activity of cyclin-dependent kinases. The cyclins are the regulatory subunits of enzymes like cyclin-dependent protein kinases (CDKs) which regulate the cell cycle in eukaryotes by means of protein phosphorylation. Auxin has been shown to regulate the expression of the gene that encodes CDC2 (cell division cycle 2) that is the major CDK. However, CDK alone is not sufficient to stimulate cell division.

In Arabidopsis tissue cultures, two G1 type cyclin proteins, viz., δ3 cyclin and δ2 cyclin have been identified. Cytokinin has been shown to stimulate the expression of the G1 cyclin gene that encodes δ3 cyclin protein, whereas sucrose, a carbon source of cultured tissues, stimulates the expression of the other G1 cyclin gene coding for the protein δ2.

Observation suggests that the culture medium should contain a combination of auxin, cytokinins and a carbon source like sucrose that is necessary for the formation of active CDK-G1 cyclin complex. In such a culture, the cells of a dormant tissue may be induced to divide and enter the cell cycle through the action of CDK-cyclin complex which permits protein phosphorylation and cell cycle regulation.

**Gibberellins (Physiological Effects in detail): (contd.)**

1. **Substitute for light in Long Day plant**

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

1. **Breaking the dormancy of Buds:**

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

1. Inhibition of Root Growth:

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

1. **Elongation of the Internodes:**

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

1. Bolting and Flowering:

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

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

1. Parthenocarpy:

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

1. Induced Light Inhibited Stem Growth:

It is common observation that the dark grown plants become etiolated and have taller, thinner and pale stems while the light grown plants have shorter, thicker and green stems, and it may be concluded that light has inhibitory effect on stem elongation. Treatment of light grown plants with gibberellin also stimulates the stem growth and they appear to be dark brown. In such cases the protein content of the stem falls while soluble nitrogen content increases prob­ably due to more breakdowns of proteins than their synthesis.

It is considered that the light in some way lowers the level of endogenous gibberellins and inhibits the stem growth.

1. De novo Synthesis of the Enzyme-α-Amylase:

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

**Mode of Action of Gibberellins**

1. **Gibberline stimulate both Cell Division and Cell Elongation:**

Stem elongation in plants as a result of gibberellin treatment involves both cell division and cell elongation. In rosette long day plants, GA treatment causes marked increase in mitosis in the sub-apical regions of apical meristems. Internodes of tall pea plants have more cells than in dwarf ones and their cells are longer in size too. In deep-water rice, stimulation of internodes elongation is partly due to increased cell di­visions in the intercalary meristems and partly due to elongation of cells of the latter who have divided with cell elongation preceding the cell divisions.

**GA Stimulated Cell Divisions are Regulated Between G2 and M Phases of Cell Cycle:**

The mechanism of GA stimulated cell division has been extensively studied in intercalary meristems of young internodes of deep-water rice. In this case, the GA – stimulated cell divisions are believed to be regulated between G2 and M phases of cell cycle. The transitions between different phases of cell cycles are known to be regulated by cyclin- dependent protein kinases (CDKs). GA stimulates cell divisions by increasing the expression of two genes (CDC2) that encode CDKs and M cyclins which are required for entry into mitosis.

1. **Mobilization of Endosperm Food Reserves:**

As mentioned earlier, the GAs cause de novo synthesis of a -amylase in cells of aleurone layer of germinating cereal grains. (The GAs synthesized by coleoptile and scutellum of the embryo are released into the starchy endosperm from where they diffuse into the cells of aleurone layer). GAs also bring about secretion of a -amylase and other hydrolytic enzymes from the cells of aleurone layer into the starchy endosperm where complex carbohydrates are hydrolyzed into simple sugars which are then trans located to growing embryo to provide energy source. Although the mechanism of induction of a-amylase synthesis in cells of aleurone layer by GA and its secretion from cells of aleurone layer into the endosperm, have been extensively studied, but they are yet far from being completely elucidated.

**Plant Responses to Cytokinins (in detail)**

Cytokinins play a prominent role in all the phases of plant development from cell division and enlargement to the formation of flowers and fruits. They increase resistance to aging and to adverse environment.

 **(i) Cell Division:**

For continued in vitro growth and cell division of tissue accompanied by DNA synthesis, cytokinin is necessary along with auxin. While auxin and gibberellin are also able to stimulate DNA synthesis and mitosis, cytokinin alone can stimulate **cytokinesis**. Quite opposite to the pro-motive effect of auxin and gibberellin, cytokinin inhibits elongation of stem sections. Root growth is generally inhibited by cytokinins.

**(ii) Cell Enlargement:**

Cytokinins may stimulate radial growth of stem tissue by swelling rather than by longitudinal extension. Vine well-known leaf enlargement caused by cytokinin is due to an effect on cell enlargement rather than cell division. In fact, cytokinin appears to promote overall enlargement of cells and not simply elongation. Cytokinin effect on cell enlargement may be due to an influence on micro fibril orientation from longitudinal to radial direction.

**(iii) Tissue Differentiation:**

Organs in tissue culture show a spectacular response to cytokinin. With a low cytokinin supply, the tissue remains as an amorphous undifferentiated callus.

Bud formation and shoot initiation depend on higher concentrations of cytokinin by changing cytokinin auxin ratios. An interesting observation on morphogenesis in tobacco callus cultures is that a high cytokinin auxin ratio results in the production of shoots but no roots, but a low ratio leads to an opposite effect producing roots only.

In addition to their role in leaf expansion, cytokinins also play a regulatory role in chloroplast formation. When cytokinin is absent, plastids are formed but remain undifferentiated. Presence of both light and cytokinin is necessary for grana development and conversion of pro-plastids into chloroplasts.

**(iv) Retardation of Senescence:**

The retardation of senescence by cytokinin is a well-known phenomenon. Richmond and Lang first discovered that when leaf discs are kept in water, senescence appears within a few days as evident by the loss of chlorophyll and protein. But when cytokinin is added to the leaf discs, senescence is delayed through the maintenance of chlorophyll and protein.

This senescence-retarding property of cytokinin as mediated through the retention of chlorophyll is known as **Richmond-Lang** effect.

**(v) Mobilization of Nutrients:**

Mothes observed that when a particular area of leaf is treated with cytokinin, that treated area remains green showing delay of senescence, while the untreated area loses its green colour and becomes yellowish showing symptoms of senescence. Here the nutrients are drawn or mobilized from other parts of leaf so that the treated area remains green at the expense of the untreated area.

**(vi) Release of Dormancy of Seeds and Buds:**

Applications of cytokinins can stimulate germination and break dormancy. One of the remarkable characteristics of cytokinins is their ability to modify the effects of other hormones without any marked effects by themselves.

When dormancy is imposed either by high temperature (thermo dormancy) or by an accumulation of inhibitor like ABA (inhibitor dormancy), then GA alone is not capable to overcome dormancy. Addition of cytokinin opposes the action of inhibitor and permits germination.

Thus cytokinin has been documented as a permissive agent in germination by antagonizing the inhibitor action — a case of cytokinin-inhibitor antagonism. In bud growth inhibitor (preventive) and cytokinin (permissive) show opposite effects. Thus inhibitor-induced bud dormancy can be overcome by cytokinin.

**(vii) Masking of Apical Dominance:**

Cytokinin applied on lateral buds is able to mask from the effect of apical dominance whether it is due to the presence of terminal bud or due to applied auxin. This has been interpreted as an increase in IAA transport and mobilization of metabolites from the apical region to the point of application of cytokinin which is supported by the striking influence of cytokinin on phloem transport.

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