**Dr. Rima Kumari: Date: 7/08/2020**

Online class and e- content for BSc IIIrd year students

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| Date and Time | Online class medium | E. content topic |
| 07/08/2020  01:30 p.m to 2.30 p.m | Via Google meet  Link: Meeting URL: https://meet.google.com/ceo-mhfd-muk | **Bioassay of Auxin, physiological function and bioassay of Gibberline and cytokinin** |

**Auxin**

**Bio-Assay test:**

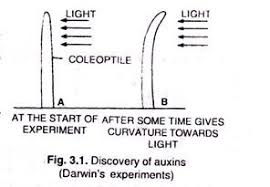
Bioassay means the testing of substance for it's activity in causing a growth response in a living plant or it's parts.

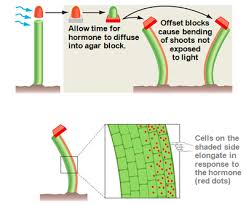
(i) **Avena curvature test**: Avena curvature test carried out by F.W. Went (1928), demonstrated the effect of auxins on plant growth by performing some experiments with the oat (Avena sativa) coleoptile.

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

**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**

**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