Plant Growth Substances /Hormones

Plant Hormones

Plants need sunlight, water, oxygen, minerals for their growth and development. These are external factors.

Apart from these, there are some intrinsic factors that regulate the growth and development of plants. These are called plant hormones or "Phytohormones".

- Plant hormones are chemical compounds present in very low concentration in plants. They are derivatives of indole (auxins), terpenes (Gibberellins), adenine (Cytokinins), carotenoids (Abscisic acid) and gases (Ethylene).
- These hormones are produced in almost all parts of the plant and are transmitted to various parts of the plant.
- They may act synergistically or individually. Roles of different hormones can be complementary or antagonistic.
- Hormones play an important role in the processes like vernalization, phototropism, seed germination, dormancy etc. along with extrinsic factors.
- Synthetic plant hormones are exogenously applied for controlled crop production

So, Plants possess a well-developed system of chemical messengers that induce (inhibit or promote) growth and developmental responses. These chemical messengers are termed "hormones". *They are **defined** as:*

1. Small;
2. Organic compounds;
3. Synthesized by the plant;
4. Active in low concentration ($<10^{-6}$);
5. Promote or inhibit growth and developmental responses;
6. Often show a separation of the site of production and the site of action. There are five major **groups** of plant hormones. These groups are:

(1) auxins;
(2) gibberellins;
(3) cytokinins;
(4) abscisic acid and
(5) ethylene.

The main functions of plant hormones?

Plant hormones control all the growth and development activities like cell division, enlargement, flowering, seed formation, dormancy and abscission.

Based on their action, plant hormones are categorized into two categories:

- Plant Growth Promoters
- Plant Growth Inhibitors

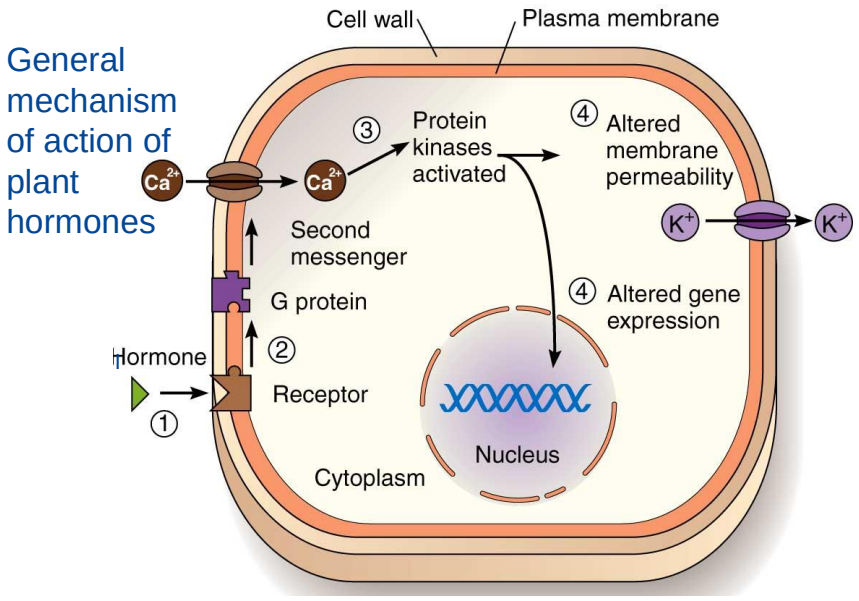
Charles Darwin first observed the phototropism in the coleoptiles of canary grass and F.W. Went first isolated auxin from the coleoptiles of oat seedlings.

Mechanism of hormone action

Hormones act on target tissues to activate a receptor. The general mechanism is: **hormone → target tissue/cell → receptor → signal amplification → response**

Thus, for a response to occur:

1. The hormone must be present in sufficient quantity;
2. The target tissue must be sensitive to the hormone;
3. The target tissue recognizes the hormone (i.e., there must be a receptor to which the hormone can bind);
4. The binding of the hormone/receptor should initiate a change in the receptor (amplification).
5. The activated receptor initiates a physiological response



Techniques to study hormones

A. Bioassays

A bioassay examines the effect of a test substance on plant tissue. To perform a hormone bioassay, a test plant is chosen that lacks the hormone for a response. Known amounts of hormone are added to the plant growth medium, the response is measured, and a "standard curve" is produced. To determine if a sample contains the hormone, the test plant is treated in a similar fashion. If present, the hormone can be quantified by comparing its response to the samples of known concentration.

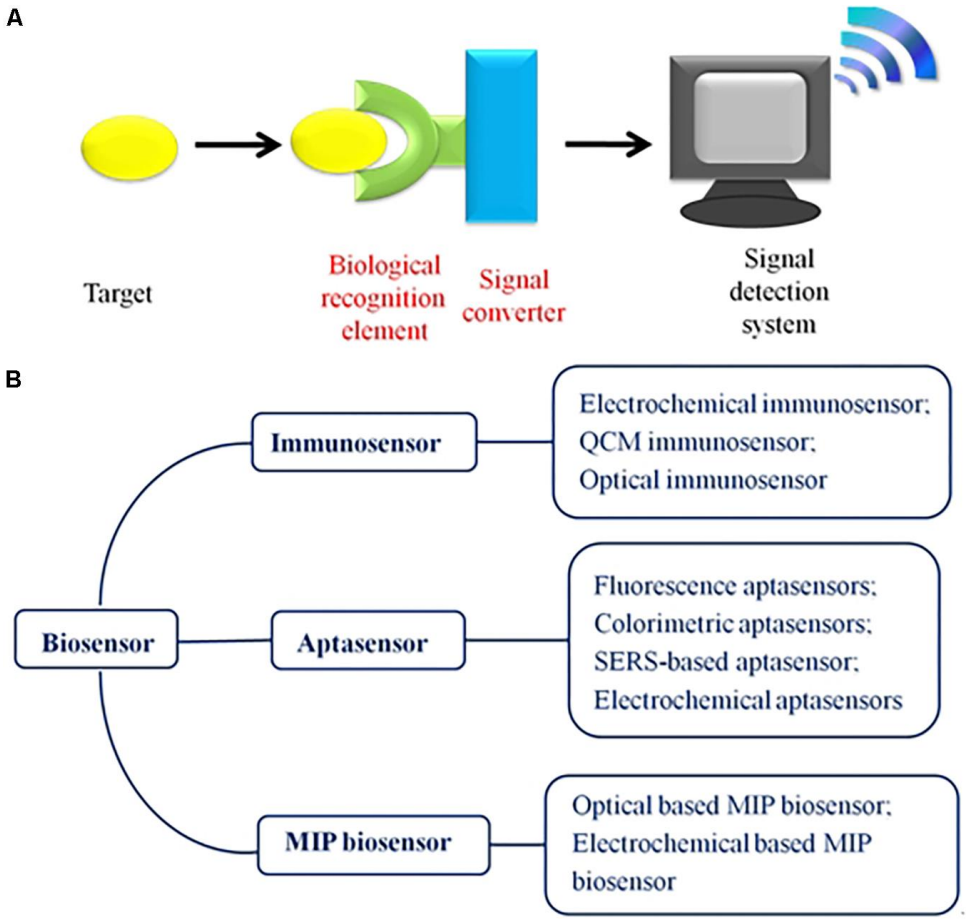
B. Immunological studies

Antibodies are made against the plant hormones and then used as specific probes to localize and quantify. The antibodies are usually coupled to radioisotopes or fluorescent dyes to make them easier to trace. Or using Bio-sensors. These techniques both are very sensitive and specific.

A biosensor is a device that measures biological or chemical reactions by generating signals proportional to the concentration of an analyte in the reaction.

So. bio-sensor is a kind of detection method used to convert biological signals into electrical signals. This detection method offers an excellent performance, as it is easy-to-use, inexpensive, very specific, and highly sensitive. Generally, a biosensor includes three main parts: a bio-recognition component, a signal converter, and a signal measurement system. The bio-recognition element is the core part of a biosensor, and

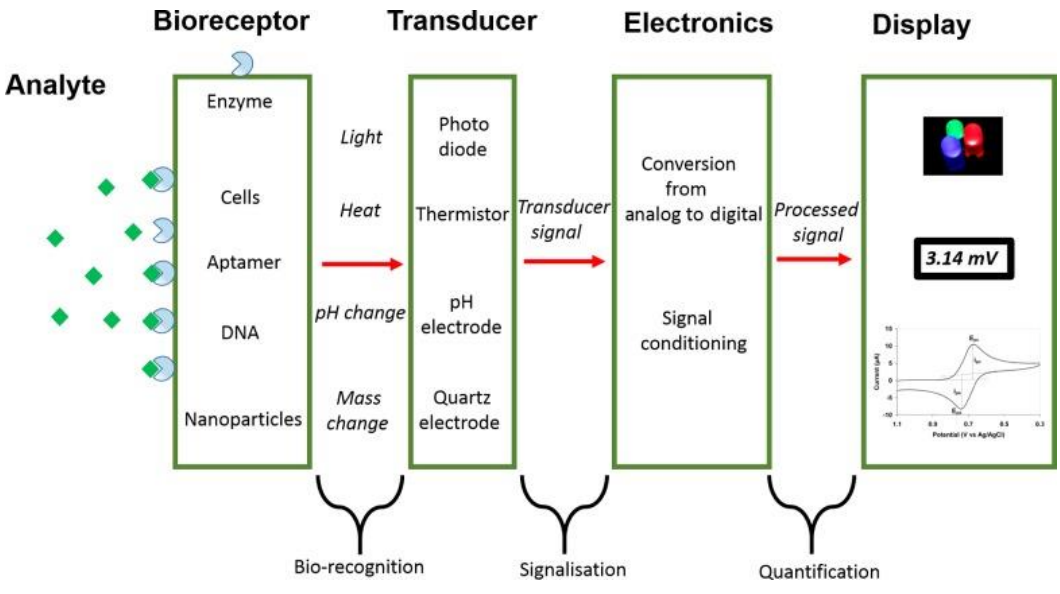
common bio-recognition elements include aptamers), antibodies, molecularly imprinted polymers (MIPs), and enzymes. These bio-recognition elements possess a high selectivity and specificity for specific target substances, and only in this way can biological sensors achieve better selectivity. In addition, the signal converter is closely connected to the biological recognition component. First, the target molecules are captured by the biological recognition component. Then, the signal converter converts the biological signals into physical signals, including electrical signals, fluorescence signals, magnetic signals, and so on. Finally, these signals are detected by the detection system. Sometimes, the signal generated by the signal converter will be amplified by the signal amplifier before reaching the detection system.



Figure/ (A) Schematic illustration of the biosensor, including the following three parts: the bio-recognition element, the signal converter, and the signal measurement system. **(B)** Outline of the biosensors used for monitoring AFB1. According to the bio-recognition element, the biosensor is divided into aptasensors, immunosensors, and MIP biosensors in this review.

Component of biosensor method:-

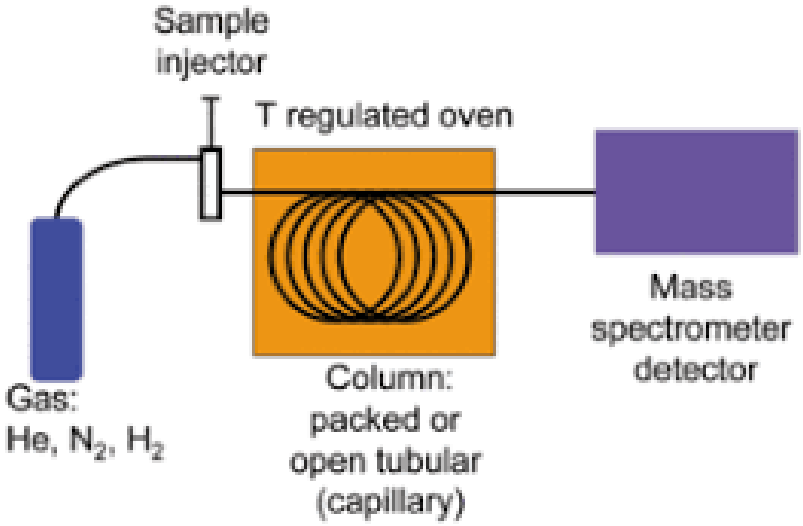
- **Analyte**: A substance of interest that needs detection. For instance, glucose is an ‘analyte’ in a biosensor designed to detect glucose.
- **Bioreceptor**: A molecule that specifically recognises the analyte is known as a bioreceptor. Enzymes, cells, aptamers, deoxyribonucleic acid (DNA) and antibodies are some examples of bioreceptors. The process of signal generation (in the form of light, heat, pH, charge or mass change, etc.) upon interaction of the bioreceptor with the analyte is termed bio-recognition.
- **Transducer**: The transducer is an element that converts one form of energy into another. In a biosensor the role of the transducer is to convert the bio-recognition event into a measurable signal. This process of energy conversion is known as signalisation. Most transducers produce either optical or electrical signals that are usually proportional to the amount of analyte–bioreceptor interactions.
- **Electronics**: This is the part of a biosensor that processes the transduced signal and prepares it for display. It consists of complex electronic circuitry that performs signal conditioning such as amplification and conversion of signals from analogue into the digital form. The processed signals are then quantified by the display unit of the biosensor.
- **Display**: The display consists of a user interpretation system such as the liquid crystal display of a computer or a direct printer that generates numbers or curves understandable by the user. This part often consists of a combination of hardware and software that generates results of the biosensor in a user-friendly manner. The output signal on the display can be numeric, graphic, tabular or an image, depending on the requirements of the end user

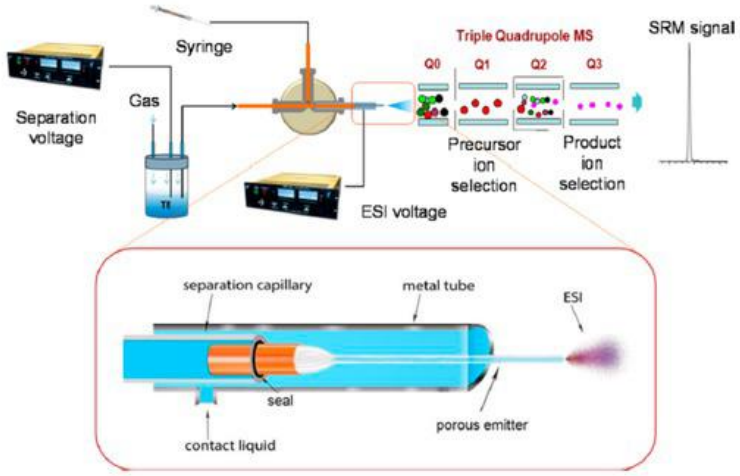


C. Instrumental Methods:

(GC-MS) Gas chromatography-mass spectrometry ; (HPLC) High-performance liquid chromatography and (Nano-flow LC-ESI-IT-MS/MS) Nanoflow liquid chromatography-electrospray ionization – ion trap mass spectrometry. high specificity and sensitivity techniques.

GC/MS is Gas Chromatography/Mass Spectrometry (GC/MS) instrument separates chemical mixtures (the GC component) and identifies the components at a molecular level (the MS component). It is one of the most accurate tools for analyzing environmental samples. The GC works on the principle that a mixture will separate into individual substances when heated. The heated gases are carried through a column with an inert gas (such as helium). As the separated substances emerge from the column opening, they flow into the MS. Mass spectrometry identifies compounds by the mass of the analyte molecule. A (library) of known mass spectra, covering several thousand compounds, is stored on a computer. Mass spectrometry is considered the only definitive analytical detector.





More recently, it has become clear that plants use additional chemical signals. The brassinolides appear to be required for the normal growth of most plant tissues. Salicylic acid has been implicated as a signal in defense responses to plant pathogens. The jasmonates are now known to act as regulators of plant development. Systemin is used in tomatoes as a long-distance signal to activate chemical defenses against herbivores.

Plant hormones and growth regulators

- Hormones
  - Auxins
  - Cytokinins
  - Gibberellins (GA)
  - Ethylene (ethene)
  - Abscisic acid (ABA)
- Other growth regulators
  - Brassinosteroids
  - Salicylic acid (SA)
  - Jasmonic acid (JA)
  - Systemin

Auxins

Introduction

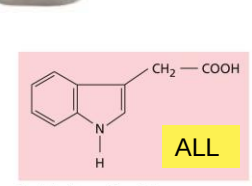
Auxin is a general name for a group of hormones that are involved with growth responses (i.e., elongate cells, stimulate cell division in callus). The term "auxin" is derived from the Greek word "to increase or grow". This was the first group of plant hormones discovered. **Chemistry/Structure**

A. Naturally Occurring Auxins:-

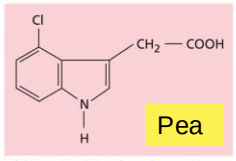
The most important auxin found in plants is indole-3-acetic acid (IAA). IAA is comprised of an indole ring linked to acetic acid.

Plant Hormone: Auxin

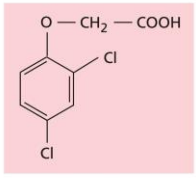
Auxin role:
http://www.youtube.com/watch?v=zctM_TWg5Ik



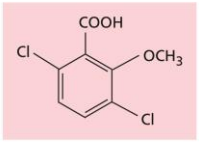
Indole-3-acetic acid (IAA)



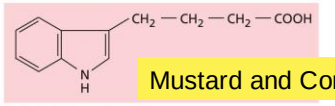
4-Chloroindole-3-acetic acid (4-Cl-IAA)



2,4-Dichlorophenoxyacetic acid (2,4-D)



2-Methoxy-3, 6-dichlorobenzoic acid (dicamba)



Indole-3-butyric acid (IBA)

PLANT PHYSIOLOGY, Fourth Edition, Figure 18.3 © 2006 Sinauer Associates, Inc.

15

B. Synthetics with Auxin Activity:-

There are a variety of substances that are not known to occur in plants that have auxin activity. These include indolebutyric acid (IBA); naphthalene acetic acid (NAA); 2,4- dichloro-phenoxy acetic acid (2,4-D), and 2,4,5-trichlorophenoxyacetic acid (2,4,5-

T). The exact mechanism of action of these compounds is not known, but they may inhibit nucleic acid synthesis.

C. Conjugated forms:-

Auxins, as do other hormones, occur in a free or conjugated (bound to sugars, alcohols, or other molecules) form. In fact, up to 98% of the auxin may be bound.

Biosynthesis

A. Site

Auxin is made in actively growing tissue which includes young leaves, fruits, and especially the shoot apex. Made in the cytosol of cells

B. Routes:- There are two major routes to the production of IAA.

1. Tryptophan-dependent Pathways. The similarity of the chemical structure of IAA and tryptophan suggested a connection between these. Considerable research has shown that tryptophan, one of the protein amino acids, is a precursor of auxin biosynthesis.
2. Tryptophan-independent Pathway - this route doesn't involve tryptophan directly as an intermediate to the formation of auxin.

Transport

Basipetal (or Polar) Transport: - Auxin is transported in a basipetal (towards the base) direction. In other words, auxin moves from the shoot tip towards the roots.

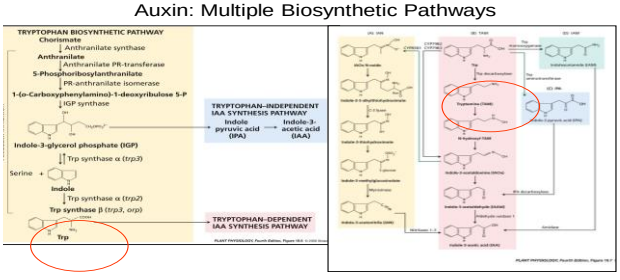
Bioassays

There are four classic bioassays for auxin. These tests, which are all based on the ability of auxin to stimulate shoot growth (or inhibit root growth), are:

A. *Avena* coleoptile curvature test:-

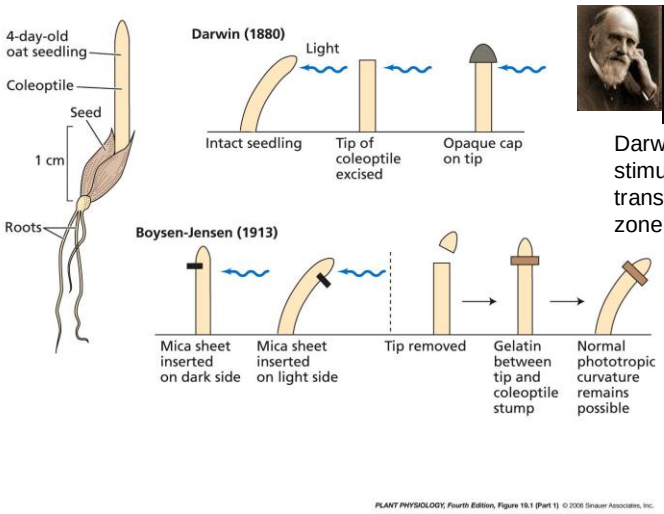
Pioneered by F. Went. The angle of curvature of a decapitated oat coleoptile is measured after placing an agar block containing auxin on one side. Then, the angle of curvature vs. [IAA] is plotted.

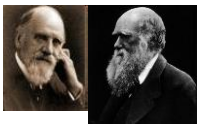
Auxin: Multiple Biosynthetic Pathways



IAN: Indole -3-acetonitrile Pathway
TAM: Tryptamine Pathway
IAM: Indole-3-acidamide Pathway
IPA: Indole-3-pyruvate pathway

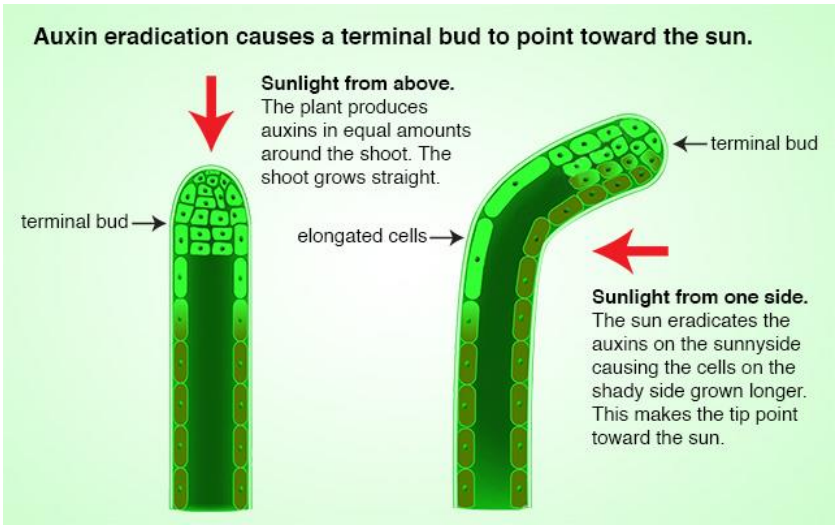
Auxin: earliest experiments

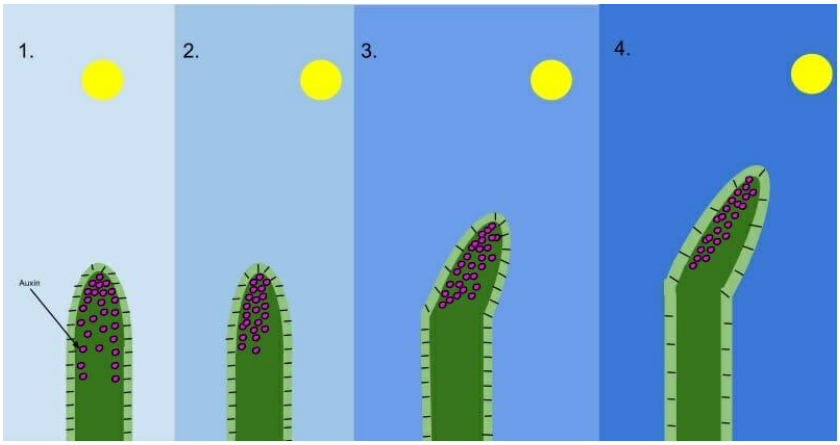




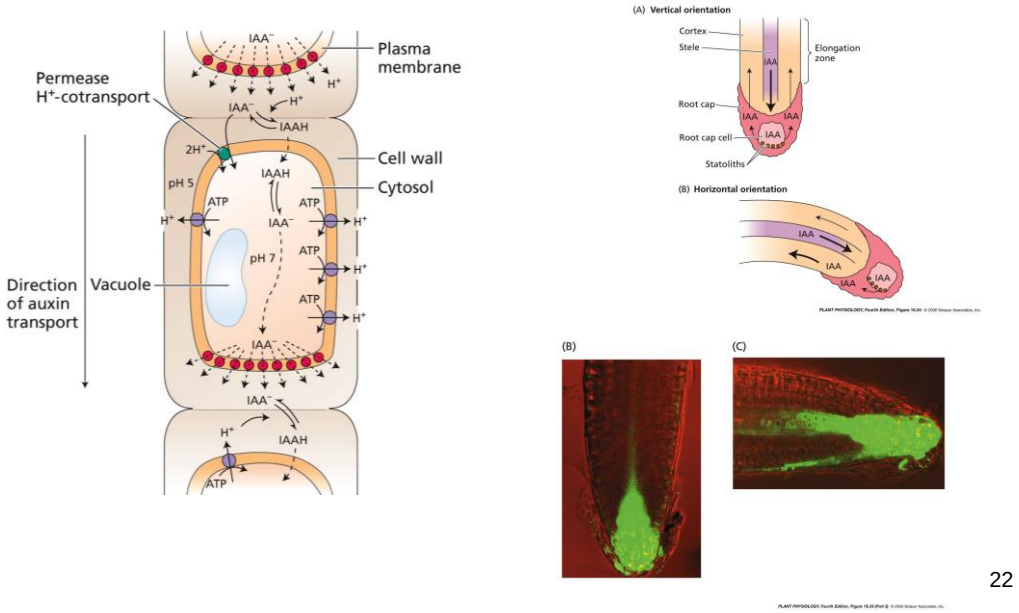
Darwins 1880, stimulus in tip and transmitted to growth zone.

Boysen-Jensen 1913, Stimulus passe through permeable membrane





Polar Auxin Transport



B. *Avena* coleoptile elongation:-

The ratio of final length/original length for oat coleoptiles or pea stem sections is measured after the tissues are floated in solutions containing different concentrations of IAA. Elongation vs. [IAA] is plotted.

C. Split pea curvature test:-

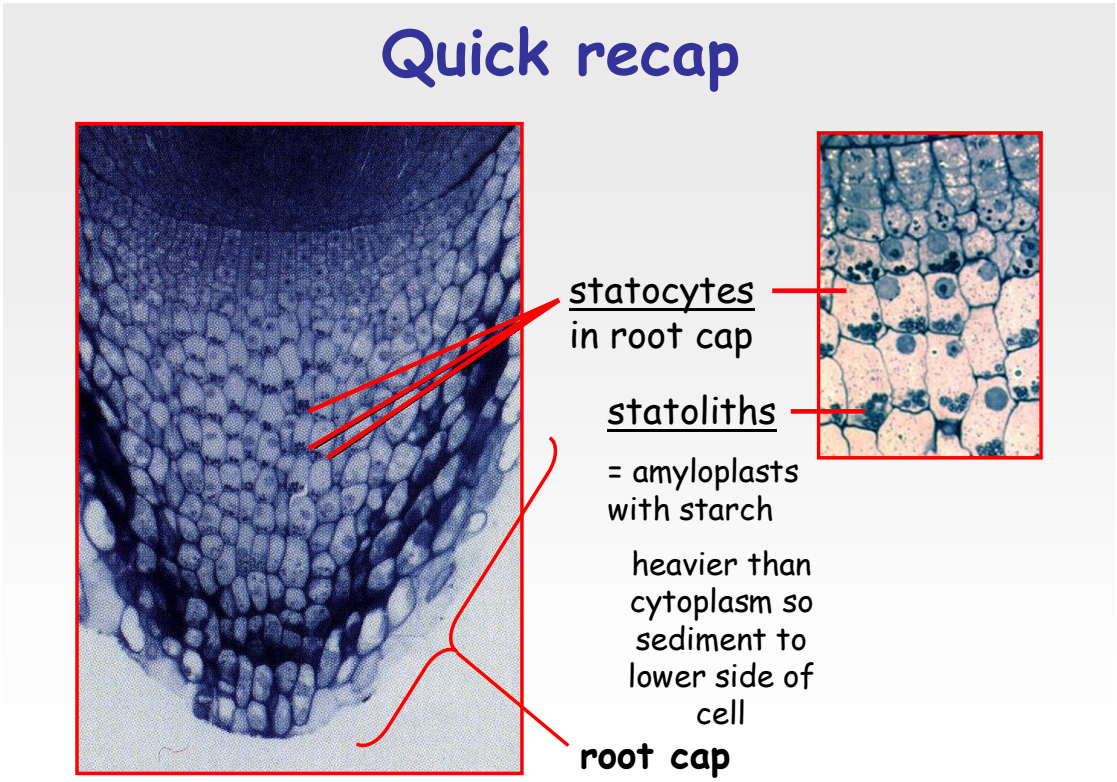
A section of pea hypocotyl is obtained and split halfway down the middle. After incubating the sections in solutions of known IAA concentration, the angle of curvature is measured. Note that only the epidermal cells are responding to the treatment.

D. Cress root inhibition:-

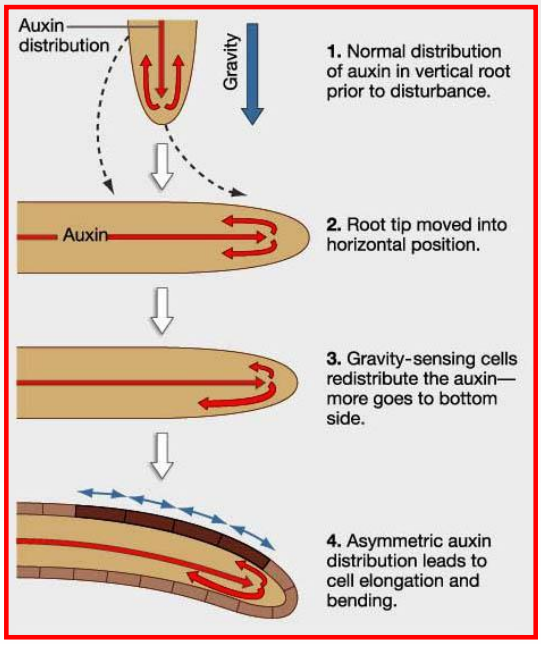
This bioassay is based on the ability of auxin to stop root growth. A ratio of treatment/control growth is plotted vs. log [IAA].

Auxin responses:-

Gravitropism is an important plant growth response to the environment that directs shoots upward and roots downward, thereby allowing each organ to reach environments that are adequate for performance of their primary functions. Gravity sensing involves the sedimentation of dense amyloplasts within specialized gravity-sensing cells in each organ. This pathway leads to the development of a lateral gradient of the phytohormone auxin across gravity-stimulated organs. Because auxin promotes cell elongation in shoots and inhibits it in roots, this gradient is responsible for an organ tip curvature that allows it to resume growth at a predefined angle from gravity.



Gravitropism mode of action



auxin moves downward in vertical root

if shifted to horizontal, statocytes have their statoliths sediment

- believe they contact membrane-bound receptor molecules

statolith movement triggers auxin redistribution to lower side of root

greater concentration of auxin on lower side of root actually inhibits cell elongation, so root bends downward

42

GIBBERELLINS

General

- "foolish seedling" disease- rice plants tall, weak and spindly with little grain - was observed early the past century by Japanese farmers.
- associated with the fungus, *Gibberella fujikuroi*.
- liquid from the culture medium applied to plants caused symptoms
- three gibberellins were isolated and identified from the medium & fungus
- gibberellins are known in angiosperms, gymnosperms, ferns, mosses, algae, fungi, and even a few bacteria.

Chemistry

- characterized by having a complex system of 4-5 rings
- more than 110 different gibberellins are known - abbreviated GA_1...GA_n.
- No more than 12 or so different gibberellins occur in any one species
- only a few (about 15) of the GA's have biological activity.

- GA_1 is the most active GA
- GA_3 was the first one discovered
- GA's can occur in a free or conjugated form

Biosynthesis

A. Site: young leaves, roots, and developing seeds (developing endosperm) and fruits.

B. Pathway

- terpene pathway
- a basic terpene building block is isoprene, a five-carbon unit

Bioassays/Analysis

A. Three common bioassays used for gibberellins are:

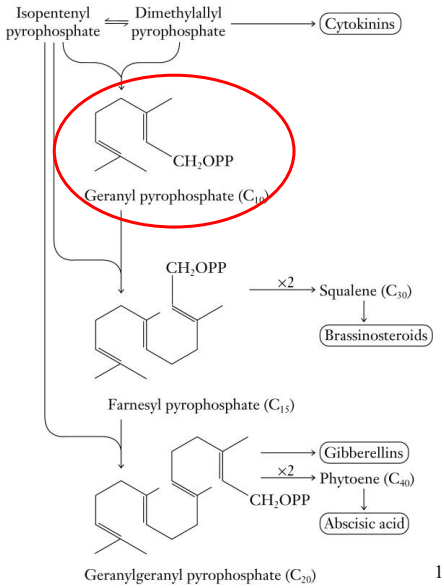
- Lettuce hypocotyl elongation
- Dwarf rice leaf sheath elongation
- alpha-amylase production in barley

B. Instrumental methods – especially GC-MS

Biosynthesis of GA, ABA, and BR

• Common precursor: geranyl pyrophosphate.

•GA, ABA and BR are terpenes (Polymers of 5-carbon unit called isoprene



17

Transport

- made in the tissue in which it is used
- transport occurs through xylem, phloem, or cell-to-cell.
- phloem seems to be the most important transport route
- transport is not polar, as it is for auxin.

Actions

A. Promotes stem elongation

When applied to intact plants, GA usually causes an increase, unlike auxin. It overcomes dwarfism in mutants that have a mutation in the GA synthesis pathway.

(dwarf = short; wild type = tall; dwarf + GA = tall). Thus, GA application: (1) stimulates elongation; and (2) acts on intact plants.

GA stimulates stem elongation by:

1. stimulating cell division. Specifically, GA increases the transition from G1 → S phase of the cell cycle;
2. increasing amylase (and other hydrolytic enzymes) production → increases
3. hydrolysis of starch → provides glucose and other sugars that: (a) lower water potential which provides a driving force for water uptake; (b) provide energy through cell respiration; (c) provide materials for building cell walls; and
4. increase cell wall plasticity (by a mechanism other than how auxin works).

B. Overcomes dormancy in seeds and buds

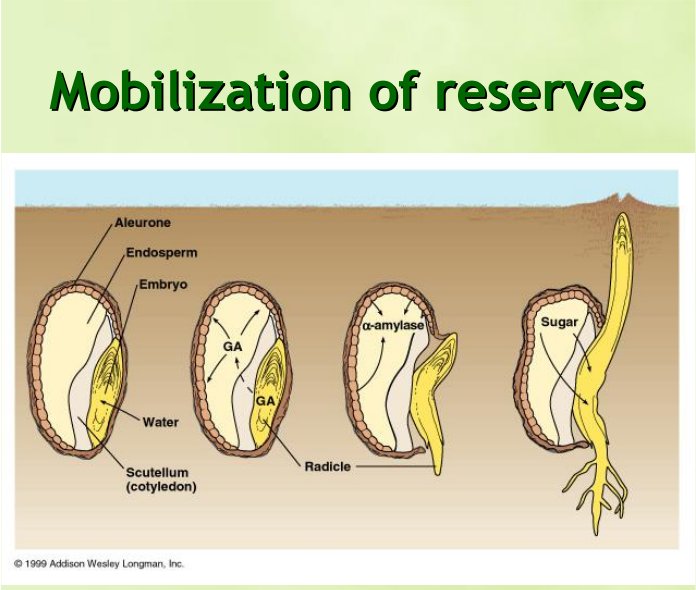
Treating dormant seeds with GA stimulates germination

C. Involved in parthenocarpic fruit development

D. Flowering

E. Mobilization of food reserves in grass seed germination

GA is produced by the scutellum (cotyledon) of the embryo → stimulates the production of amylase by the aleurone layer → amylase hydrolyzes starch to simple sugars →absorbed by scutellum and translocated to embryo for growth.



F. Juvenility

Plants exist in juvenile and adult forms. As in humans, the main difference is whether the plants can flower (reproduce). In some plants, there is little morphological difference between juvenile and adult forms, whereas in others, the two forms are very distinct. For example, in beans, the first (juvenile) leaves are entire (heart-shaped) while the adult leaves are trifoliate. Gibberellin stimulates the transition between the juvenile and adult forms. In ivy, the adult form (unlobed leaves, shorter internodes) is converted to the juvenile form (lobed leaves, longer internodes) by GA treatment.

G. Sex expression

In plants with separate male and female flowers, GA application can determine sex. For example, in cucumber, hemp and spinach, GA treatment increases the proportion of male flowers. In maize, GA treatment causes female flower development.

Commercial Applications

- increase size of grapes (spray at time of blooming and fruit set stage)
- increase distance between grapes in a cluster to minimize fungi/disease
- Delay senescence - spray on fruit like oranges
- Minimize lodging.

Cytokinins

General

- Called "cytokinins" because they stimulate cell division (*i.e.*, cytokinesis)
- Haberlandt (1904) noted that non-dividing potato parenchyma cells would revert to actively dividing ones in the presence of phloem sap. This observation suggested a soluble material was responsible for cell division.
- Folke Skoog (1940's) and colleagues at the Univ. of Wisconsin found that cultured tobacco pith tissue explants would proliferate only if they were supplemented with various substances such as autoclaved herring sperm or coconut milk.
- Miller (1956) identified the first cytokinin, called kinetin, in the herring sperm.
- Cytokinins occur in most plants including mosses, ferns, conifers, algae and diatoms

Chemistry

A. General

- adenine derivatives (amino purines)
- approximately 40 different structures known.
- Zeatin (Z), which was first isolated from maize (*Zea mays*) is the most common cytokinin.
- Other naturally occurring cytokinins include dihydrozeatin (DHZ) and isopentenyladenosine (IPA).

B. Synthetic cytokinins

- kinetin : probably byproduct of zeatin degradation
- there are several other substances with cytokinin activity such as benzyl adenine (benzylaminopurine; BA).

Synthesis

- Site: synthesized primarily in the meristematic region of the roots. This is known in part because roots can be cultured (grown in an artificial medium in a flask) without added cytokinin, but stem cells cannot.
- Cytokinins are also produced in developing embryos and crown gall tissues

Transport

- via xylem (transpiration stream)
- in peas, a signal from the leaves may signal/regulate the transport of cytokinins from the roots
- Some cytokinin also moves in the phloem.

Bioassays/Analysis

A. Bioassay

1. Callus culture cell proliferation
2. Expansion of radish cotyledons
3. Inhibition of chlorophyll loss by detached oat leaves during senescence

B. Methods of Analysis – liquid chromatography, mass spectroscopy; radioimmunoassay.

Actions

A. Control morphogenesis

- In plant tissue cultures, cytokinin is required for the growth of a callus (an unorganized, tumor-like mass of cells):
  - Callus + auxin → little growth of callus
  - Callus + auxin + cytokinin → callus grows well, undifferentiated
- The ratio of cytokinin and auxin are important in determining the fate of the callus:

callus + low [cytokinin/auxin] → callus grows well, forms roots

callus + high [cytokinin/auxin] → callus grows well, forms meristem & shoots

- Some tissues become habituated during repeated cell culture – lose the requirement for cytokinin in the growth medium .

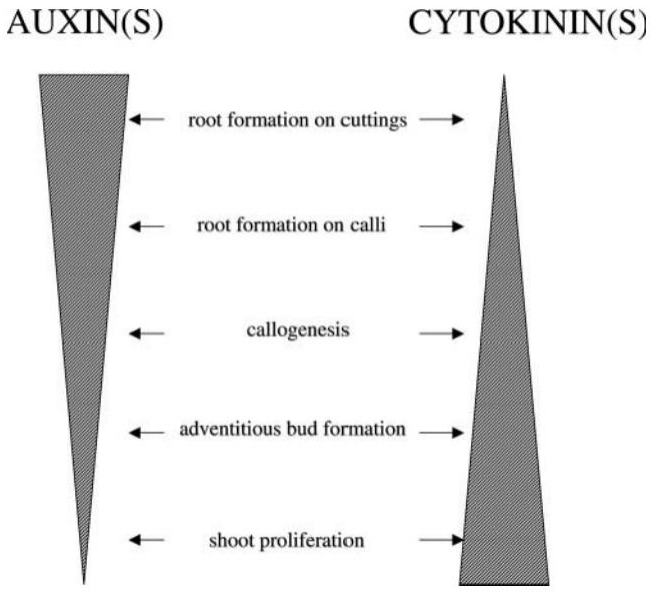


Figure shows the control of different organogenic programe by the balance between auxins and cytokinins.

B. Regulates the cell cycle/cell division (hence, the name "cytokinins)

C. Delay senescence

- senescence is the programmed aging process that occurs in plants (and other organisms for that matter).
- loss of chlorophyll, RNA, protein and lipids.
- cytokinin application to an intact leaf markedly reduces the extent and rate of chlorophyll and protein degradation and leaf drop
- correlation between cytokinin levels and senescence.

D. Greening

Promotes the light-induced formation of chlorophyll and conversion of etioplasts to chloroplasts (greening process).

E. Promote lateral bud development

Cytokinin application to dormant buds will cause them to develop. A witches' broom is caused by the pathogen Agrobacterium *tumefaciens*) that produces cytokinin which, in turn, stimulates lateral bud development (branching). These results suggest that apical dominance may be related to cytokinin, too.

F. Promote cell expansion

The mechanism is associated with increased plasticity of the cell wall.

Mechanism of action

Specific binding sites (receptors) for cytokinin are known. These may be ribosomal proteins. Thus, regulate protein synthesis.

Abscisic Acid (ABA)

General

- Bennet-Clark and Kefford (1953) described the presence of an inhibitor of coleoptile elongation in oats
- about 10 years later, Addicott *et al*. found a substance that stimulated the abscission of fruits in cotton and they named it abscisin II
- about the same time, Wareing found a substance in leaves that promoted dormancy in buds and called it dormant
- it was soon clear that these were the same substance
- a conference in 1967 straightened out the name and it was decided to call the hormone abscisic acid (ABA).

Chemistry

- a single structure, not a family of related structures like the gibberellins
- occurs as *cis* form.
- found in all green plants, also in some mosses, algae, and fungi

Biosynthesis

- synthesized in plastids
- most tissues, especially leaves and seeds.
- comes from intermediates of glycolysis
- ABA is derived from the breakdown of carotenoids.

Bioassays/Analysis

A. Bioassays – there are several including:

- inhibition of seed germination
- inhibition of GA induced alpha-amylase production

B. Analysis – Gas chromatography, HPLC, and immunoassay

Disposal/Regulation

- rapid changes in endogenous levels (up to 100x within a few days)
- ABA levels can be regulated by: (a) degradation; (b) compartmentalization; (c) transport; (d) conjugation to sugar or another molecule.

Transport

xylem and phloem (greater amounts)

Actions

A. Growth Inhibitor
Widespread growth inhibitor; often antagonistic of GA actions

B. Maintains or "seals in" bud and seed dormancy (i.e., prevents germination)
In fact, ABA is made during the terminal stages of embryo development. Among its roles in seed dormancy is to: (1) provide desiccation tolerance of the embryo; and (b) promote the accumulation of seed storage proteins.

C. Prevents vivipary
Development of the embryo without a dormant period. Some evidence: viviparous mutants have reduced ABA

D. Inhibits auxin-induced growth

E. Stomatal closure under water stress

F. Abscission & senescence

Mechanism of action

A. Effects on the plasma membrane

B. Inhibits protein synthesis

C. Regulation of genes (transcription)

Ethylene

General

- the Chinese may have been the first to observe the effects of ethylene when they noted that burning incense increased fruit ripening
- in 1864 leaks in gas lights in street lamps were reported to stunt plant growth and defoliate trees
- in 1901, D. Neljubow realized that his dark-grown pea seedlings were short, fat, and negatively gravitropic (i.e. **the triple response**) because of a component in "laboratory air" which he subsequently identified as ethylene
- Cousins (1910) first reported that ethylene occurred in plants.

Chemistry

- single compound (like ABA) and is not a family of related ones (*i.e.*, gibberellins)
- $CH_2=CH_2$
- ethylene (MW 28) is similar in size/shape to water
- a gaseous plant hormone.

Biosynthesis

A. General:

- made by most plants including angiosperms, gymnosperms, ferns, mosses,
- also synthesized by fungi and bacteria
- made by all parts of the plant
- meristematic regions (shoot apex) and senescing tissues are rich sources
- nodes make more ethylene than internodes
- ethylene production is stimulated by physiological stresses including wounding, anaerobic conditions, flooding, chilling, disease and drought.

Actions

A. Fruit ripening

Ethylene triggers fruit ripening.

B. Abscission

This is the shedding of plant parts. Occurs at a specialized layer of cells – the abscission layers.

C. Epinasty

Downward bending of leaves - common response to flooding or waterlogged soils.

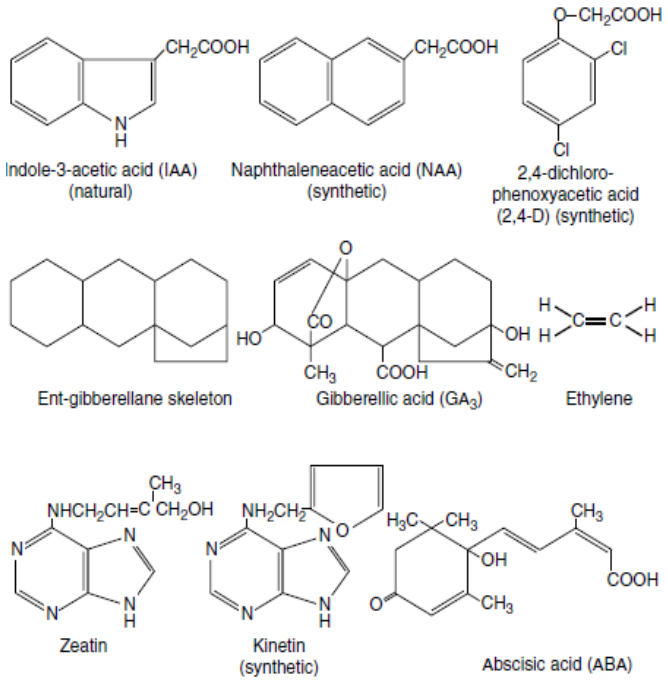
D. Triple Response

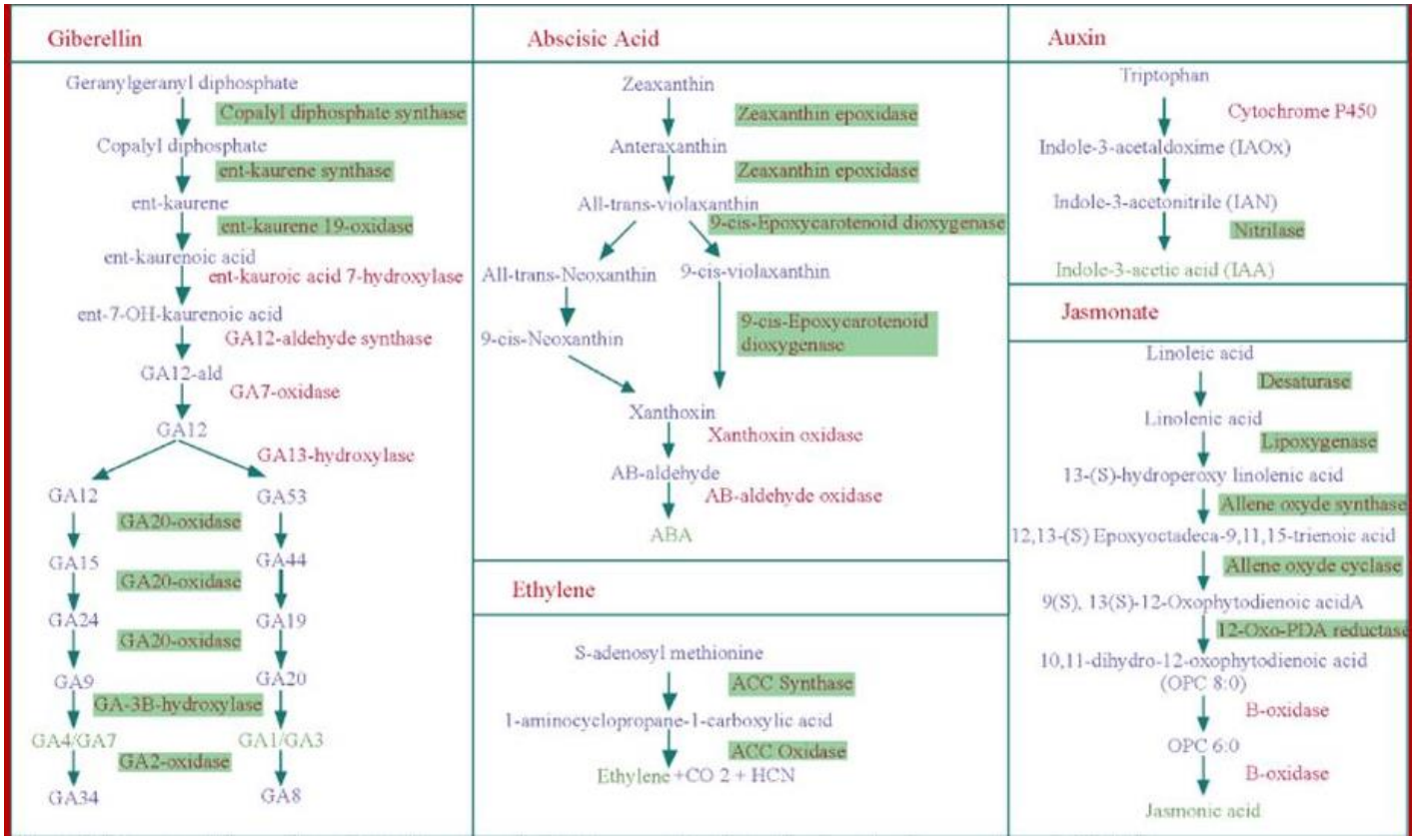
Pea seedlings treated with ethylene are short (inhibits internode elongation), fat (increase stem thickness) with negative gravitropism. Further, they show little leaf expansion and possess an apical hook.

E. Stimulates germination in cereals, peanuts.

F. promotes sprouting in potato tubers and other bulbs.

G. Flower senescence





Very small concentrations of these substances produce major growth changes.

Compound	Effect/Use
Gibberellic acid (GA)	Stimulates cell division and elongation, breaks dormancy, speeds germination
Ethylene gas (CH_2)	Ripening agent; stimulates leaf and fruit abscission
Indoleacetic acid (IAA)	Stimulates apical dominance, rooting, and leaf abscission
Indolebutyric acid (IBA)	Stimulates root growth
Naphthalene acetic acid (NAA)	Stimulates root growth, slows respiration (used as a dip on holly)
Growth retardants (Alar, B-9, Cycocel, Arest)	Prevent stem elongation in selected crops (e.g., chrysanthemums, poinsettias, and lilies)
Herbicides (2,4-D, etc.)	Distorts plant growth; selective and nonselective materials used for killing unwanted plants