

PLANT REGULATORS

A. Historical background

1. The presence of hormones in plants was first suspected by Charles and Francis Darwin when they studied the effect of light and gravity on the bending of oat seedlings
 - a. their observations on the response to light were:
 - 1) intact oat seedlings bent toward unilateral light
 - 2) removal of the coleoptile tip stopped the bending response
 - 3) covering the tip stopped the bending response
 - 4) light reception and the bending response could be separated in time
 - 5) the bending response travelled downward
 - b. in 1880 they published their work in a book called "The Power of Movement in Plants". On the effect of light they said, "When seedlings are freely exposed to a lateral light some influence is transmitted from the upper to the lower part causing the latter to bend." On the effect of gravity they stated, "...it is the tip alone which is acted on, and ...this part transmits some influence to the adjoining parts, causing them to curve downwards."
2. Eventually the "influence" was shown to be a

chemical substance called an "auxin" (indole acetic acid - IAA)

3. Fitting (1909) found that water extracts of pollen caused swelling of flower pistils. (Saliva caused a similar response)
4. Boysen-Jensen (1913) found that if a piece of mica was inserted into the dark side of a coleoptile just below the tip the plant would not bend toward the light, but if the mica was inserted on the lighted side the plant would bend. Also, he found that the substance moving downward moved through an agar block.
5. Kogl, Haagen-Smit and Erxleben (1930's) were able to isolate enough of the chemical from the urine of pregnant women to characterize it. At first they identified a compound they called auxin a. They were never able to isolate it again. Instead, they identified IAA (indoleacetic acid).
6. Many substances were tested for hormone activity from the late 20's to early 50's. Much confusion existed about terminology so in 1954 the ASPP called a conference together to define terms. In this meeting the following definitions were developed:
 - a. Plant regulators are organic compounds, other than nutrients, which in small amounts promote, inhibit or otherwise modify any physiological

- process in plants.
- b. Growth regulators are regulators which affect growth.
 - c. Flowering regulators are regulators which affect flowering.
 - d. Plant hormones are regulators produced by plants, which in low concentrations regulate plant physiological processes. Hormones usually move within the plant from a site of production to a site of action.
 - e. Growth hormones are hormones which regulate growth.
 - f. Flowering hormones are hormones which initiate the formation of floral primordia, or promote their development.
 - g. Auxin is a generic term for compounds characterized by their capacity to induce elongation in shoot cells. They resemble indole-3-acetic acid in physiological action.

B. AUXINS

1. Defn: regulator which induces elongation of shoot cells. They resemble indoleacetic acid in physiological action.
2. Structure (slide or diagram)
 - a. four classes
 - 1) indole acids
 - a) indoleacetic - IAA
 - b) indolebutyric - IBA (Rootone)
 - c) indolepropionic - IPA
 - d) 4-Chloroindole acetic acid (4-chloroIAA)
 - 2) naphthalene acids (naphthalene acetic acid - NAA)
 - 3) phenoxy acids (2,4-D; 2,4,5-T)
 - 4) benzoic acids
 - a) Phenyl acetic acid (PAA)
 - b) Trichlorobenzoic acid
 - b. activity and persistence increase as you come down the list
 - c. IAA, IBA, 4-chloroIAA and PAA are the only hormones
 - d. most phenoxy and benzoic acids are herbicides
 - e. all have ring, acidic side chain and a certain 3-dimensional structure
3. Concentration in plants
 - a. amount in oat coleoptile tip - 10^{-10} grams
 - b. bioassays needed to detect it

- 1) oat coleoptile bending test
- 2) oat coleoptile section elongation test
- 3) pea split stem test

4. Synthesis

- a. mostly produced in meristems (mass and diffuse) but also in leaves, flowers and fruits especially when enlarging
- b. pathway:

tryptophan ----> indolepyruvic acid---->

indoleacetaldehyde ----> indoleacetic acid

- c. Zn necessary for synthesis

5. Transport

- a. IAA transported readily
- b. Translocated through phloem from source to sink.

- c. Polarized transport
 - d. Active transport involved
 - 1) slower through older parts
 - 2) slower at different stages
 - a) spinach rosette stage -- poor
 - b) spinach bolting stage -- good
6. Mode of action
- a. site unknown (cytoplasm?)
 - b. increased hemicellulose and pectin production in cell wall
 - c. increased cell wall plasticity
 - d. increased water uptake
 - 1) increased osmotic potential? (hydrolysis of starch)
 - 2) active uptake?
 - e. membrane permeability changes
 - f. decrease in protoplasmic viscosity
 - g. increased protoplasmic streaming
 - h. increased respiration
 - i. alteration of nucleic acid metabolism
 - j. increased hydrolysis of starch
 - k. mitosis
7. Response pattern (graph)
- a. low concentrations promote, higher concentrations inhibit
 - b. roots more sensitive than stems or buds
 - c. two-point attachment theory

8. Plant responses influenced by auxins
 - a. stem elongation
 - b. root elongation
 - c. phototropism
 - d. geotropism
 - e. apical dominance
 - f. epinasty
 - g. root initiation
 - h. leaf expansion
 - i. development and action of phloem (direction of movement)
 - j. activation of cambial cells (diffuse meristems)
 - k. bud differentiation
 - l. flower initiation
 - m. fruit and embryo development
 - n. parthenocarpy
 - o. abscission of leaves, flowers and fruits
 - p. formation of callus in tissue cultures
 - q. herbicide

9. Inactivation or degradation
 - a. formation of inactive complexes
 - b. enzymatic degradation
 - 1) IAA oxidase breaks IAA down
 - 2) Cu an activator
 - 3) Mn also involved
 - 4) light a factor also
 - c. bound on sites (proteins, etc)

C. GIBBERELLINS

1. History

- a. Discovered by Kurosawa (1926) while studying crazy seedling disease of rice caused by *Gibberella fujikuroi*
 - 1) plants were tall and spindly
 - 2) stems too weak to support plant
 - 3) plants often devoid of fruit
- b. culture extracts of the fungus caused growth of corn and rice seedlings
- c. the chemical was isolated in 1935 and named gibberellin
- d. some of the results were published in the west but were ignored until after World War II (1950)
- e. the fungus was cultured in large quantities allowing easy access to the chemical.

2. Concentration in plants

- a. 10^{-7} to 10 ug/plant
- b. bioassays used
 - 1) dwarf corn
 - 2) dwarf pea
 - 3) rice (most sensitive - 10^{-7} to 10^{-4} M)

3. Structure (diagrams)

- a. many different forms isolated
 - 1) from fungus: GA2, GA3, GA4, GA7, GA9, etc.
 - 2) from plants, GA5, GA6, GA8, etc.

- 3) from both: GA1, etc?
 - b. GA3 the most widely used one
 - c. all have basically the same structure (five rings)
 - d. all are acidic
3. Synthesis
- a. major sites are roots and embryos in germinating seeds
 - b. complex pathway
 - c. terpene precursors
4. Mode of action
- a. cell division
 - 1) mitosis
 - 2) in subapical regions of shoot meristems
 - 3) cambium
 - 4) fruits
 - b. cell elongation
 - c. high concentrations do not inhibit as in auxins
5. Responses influenced by gibberellins
- a. increase internode length
 - 1) dwarf plants
 - a) plants grow to height of normal plants
 - 2) intact plants not just sections of plants
 - b. stimulates monocot leaf elongation
 - c. inhibits root initiation
 - d. can substitute for cold requirement
 - 1) seed germination
 - 2) tree buds (e.g. peach)

- 3) flowering in some biennials (vernalization)
 - a) called bolting
 - b) most winter grain races don't respond
 - c) reduces time needed for seed production
- e. substitutes for rest period in potato
 - 1) potato has low GA when harvested
- f. replaces light requirement needed for lettuce and tobacco seed germination
- g. stimulates synthesis of amylase in germinating seeds
- h. induces flowering in long day plants under short day conditions
 - 1) variety of effects with short day plants
- i. stimulates formation of staminate flowers in some plants
 - 1) auxins favor formation of pistillate flowers
- j. induces male flower sterility in hybrid corn
- k. may alter sex of some flowers
- l. induces parthenocarpy
 - 1) effective on grape and tomato
 - 2) doesn't have to be applied to the flower as with auxins
- m. stimulates fruit set (tomato -- spray product)
- n. stimulates fruit enlargement (grapes)

D. CYTOKININS

1. History

- a. Wiesner (1892) suggested the presence of a chemical substance which would control cell division.
- b. Van Overbeek (1941,1942) found that coconut milk stimulated division.
- c. Jablonski and Skoog (1954) diffusates from vascular cells stimulated cell division in tobacco pith cultures
 - 1) pith forms callus which soon stops growing
 - 2) IAA stimulates early growth but this soon stops also
 - 3) callus transferred to fresh medium does not grow
 - 4) coconut milk or yeast extract stimulated new division and growth when IAA was present
 - 5) tests showed that the active fraction was DNA not RNA
 - 6) fresh DNA did not work but older or autoclaved DNA did work so it was determined that the active substance was a breakdown product of DNA (adenine compound)
- d. Miller (1955) substances isolated from yeast DNA extracts. Named it kinetin.
- e. later renamed cytokinetin to avoid confusion with a different compound

2. Structure (diagram)
 - a. some naturally occurring (zeatin,
 - b. some are synthetic compounds (
 - c. are nonacidic compounds

3. Concentration in plants
 - a.
 - b. bioassays
 - 1) cell division of carrot root
 - 2) cell division of tobacco pith
 - 3) bud formation of tobacco pith
 - 4) leaf enlargement
 - 5) chlorophyll retention in leaves

4. Synthesis
 - a. major sites are root apex, germinating seeds, developing fruits

5. Transport
 - a. is not easily transportable

6. Biological effects
 - a. stimulates cell division - cytokinesis
 - 1) cytokinesis
 - 2) auxins and gibberellins influence mitosis and chromosome duplication
 - 3) cytokinins play secondary role to auxins in DNA and RNA formation
 - b. stimulates shoot initiation in callus cultures

- 1) if the ratio of auxin to cytokinin is low, leafy shoots originate
 - 2) if the ratio of auxin to cytokinin is high, roots originate
- c. stimulates leaf expansion
- 1) even in mature leaves
- d. stimulates mobilization of nutrients in leaves
- e. can break dormancy
- 1) promotes germination of light-sensitive lettuce (and some other) seeds (may increase the effectiveness of light)
 - 2) replaces the winter rest period of some plants (Hydrocharis)
- f. releases apical dominance (counteracts auxin)
- g. suppresses senescence
- 1) maintains high content of protein, RNA, and chlorophyll in leaves
- h. may inhibit root elongation
- i. auxins greatly magnify cytokinin effects

E. ETHYLENE

1. History

- a. Wiesner (1878) found that dark-grown seedlings bent over to the horizontal--thought that it was caused by the darkness
- b. Molisch (1884) reported on the effects of illuminating gas on root geotropism.
- c. Neljubow (1901) found that traces of illuminating gas in lab air caused horizontal bending.
 - 1) ethylene and acetylene were the active fractions
 - 2) described the response of plants to ethylene (triple response)
 - a) reduction of elongation (pea)
 - b) increased radial expansion (Vicia)
 - c) horizontal orientation (regardless of plant placement and requires darkness)
 - 3) responses he failed to note
 - a) inhibition of leaf or terminal bud expansion
 - b) tightening of plumular hook

2. Concentration in plants

- a.
- b. bioassays
 - 1) triple response of peas and potato sprouts
 - 2) epinasty of leaf petioles

3. Synthesis
 - a. applied auxin (2,4-D or IAA) and GA stimulate ethylene production
 - b. regions of high endogenous auxins generally are high in ethylene production (e.g. lower sides of horizontal roots and stems)
 - c. high in stems of germinating seedlings
 - d. high in ripening fruits

4. Responses in plants
 - a. stimulates epinasty of leaves
 - 1) auxin may produce ethylene
 - b. high levels suppress elongation of apical portions of etiolated seedling stems
 - 1) light reduced this effect (reduced ethylene production)
 - c. influences apical dominance
 - 1) auxin may stimulate ethylene production
 - d. shortens rest period in potato
 - e. increases stem diameter in pea seedlings
 - 1) increased mechanical inhibition of seedling stimulates ethylene production
 - 2) increases mechanical strength and emergence force of the stem in hard-packed soil
 - f. influences dormancy in light sensitive seeds (lettuce)
 - 1) red light stimulates ethylene production and breaking of dormancy
 - 2) far red light induces dormancy which can

be overcome with ethylene

- g. reduces geotropism
- h. tightening of plumular hook
- i. inhibition of leaf or terminal bud expansion
- j. stimulates leaf abscission
- k. stimulates flowering in some plants (e.g. pineapple)
- l. involved in flower physiology
 - 1) reproductive parts of flower produce more ethylene than petals and sepals
 - 2) pollination and IAA treatment stimulated ethylene production
 - 3) fading, infected or wounded flowers show high rate of production
- m. stimulates ripening in mature but unripe fruits
 - 1) self-ripening fruits (banana, apple, etc) produce ethylene during ripening
 - 2) O₂ needed and not too much CO₂
 - 3) pathway?

ethylene

mature fruit -----> ripening -->

self

ethylene production --> ripening

F. ABSCISIC ACID

1. Chemistry

a. diagram

2. Historical background

a. first isolated as dormancy-causing growth inhibitor--called Dormin

b. other researchers working on abscission-accelerating substance--called theirs Abscissin

c. found to be the same compound

3. Physiological role

a. dormancy

1) dormant buds produce ABA - stimulates dormancy in nondormant plants

2) influences dormancy in onion bulbs and potato tubers

3) maintains seed dormancy

a) fruit ABA levels higher than seed (probably does not inhibit seed germination)

b) seed content of ABA declines with cold treatment (stratification)

- c) embryo and endosperm can synthesize ABA
 - d) coleoptile inhibited more than root
 - e) GA and cytokinins counteract ABA effect on dormancy (effect varies with plant type - GA alone works in cereals but GA & cytokinins needed in lettuce))
- b. root initiation
- 1) ABA stimulates lateral root initiation
- c. abscission
- 1) leaf abscission
 - a) not sure if it is important in intact leaves
 - b) ABA found in older leaves and leaves treated with defoliant
 - 2) fruit abscission
 - a) involved in abortion of young fruits
 - b) accelerates abscission in mature fruits (peach, olive, citrus, etc)
 - c) rises and falls in cotton fruits in response to internal changes - e.g. rises with drop of young fruits, drops, then rises with fruit maturation and embryo dormancy
- d. Wilting and stomatal movements
- 1) ABA content rises in wilted leaf tissue - importance?

- 2) ABA stimulates stomatal closure -
cytokinins slightly reverse this
- e. Inhibition of growth
- 1) ABA inhibits all plant growth - whole plants, excised organs, seeds
 - 2) very potent effect
 - 3) effect can be overcome by leaching out the ABA or removing the source
- f. Senescence
- 1) ABA stimulates leaf senescence (initiates)
 - 2) Cytokinins counteract this effect
- g. Protein synthesis
- 1) ABA prevents formation of mRNA for producing amylase in seeds
 - 2) ABA may lead to production of new proteins (transcriptional control)