ABSCISIC ACID

Discovery

For a balance and coordinated growth and development , it is necessary that there should be some mechanism in plants to check growth with space and time . Presence of inhibitory substances in plants was known for a long time . Many of these substance are phenolic in nature . These are secondary plant products which do not appear to have any specific hormonal role in plants . Secondly , these are not universal in occurrence and are also not active in minute amounts to qualify as hormones .

Many plant tissue are shown to contain a group of inhibitory substances which would separate on the chromatogram with an Rf of 0.7 to 0.9 . These are called β-complex inhibitors . The active component of this group was not established till recently . It is , however , known that hormonal substances are involved inhibition of growth , dormancy , and abscissin . There groups of investigators working independently and almost simultaneously were successful in isolating the active principle from different sources .

It is of common knowledge that in cotton a large number of developing fruits abscise before attaining maturity . The cause of immature boll shedding was investigated by Addicott and his co-workers. From the dry fruit wall (burs) of cotton (*Gossypium hirsutm*) a crystalline substance was extracted (Lui and Carns 1961). This substance could induce abscission when applied to debladed petioles of cotton seedlings . It was , therefore named abscisin . A second substance was also extracted in a crystalline form by them from young cotton fruits . This was also show to be potent accelerator of abscission . It was named abscisin II and the substance reported earlier was called abscisin I (Ohkuma et al 1963). A bscisin I and II were , however , different substances . Absicsin II could not only promote abscission but also strongly inhibit growth of coleoptiles of *Avena* .Further work was done with only abscisin II .

The apical bud of many plants such as *Acer, Betula* and others becomes dormant every year with the onset of autumn and resumes growth in the subsequent spring season . It was presumed that dormancy is due to the presence of inhibitors . However , the clear cut experimental proof for this was supplied by Wareing and his associates . Their experiments suggested that an inhibitory substance is synthesized in the mature leaves of *Acer* and *Betula* during the prevailing short days of autumn . This inhibitor was shown to more upwards in the phloem and reach the growing tip and cause cessation of cell division and growth , i. e. the bud has become dormant . Finally they were able to isolate , purify and crystallize a very potent substance from the leaves of *Acer* plants grown in short days . This substance was called dormin (Robinson et al 1963) .

Both the physical and chemical properties of dormin were found to be the same as those of abscisin II (Cornforth et al 1965). Therefore , it was concluded that these two substance are identical and refer to one and the same substance . The accepted common name for both the substance is a bscisin acid (ABA).

Anther crystalline substance was extracted from the fruits of yellow lupin (*Lupinus luteus*) (Rothwell and Wain , 1964). This substance could also cause abscission and inhibit growth of the coleoptile. Lupin inhibitor was also shown to be ABA .

Detection and Estimation

Abscisic acid is detected and estimated by gas chromatography coupled with electron captor detection . This method is reliable and widely employed . Abscisic acid is also bioassay by making use of its property of inhibition of growth , stimulation of abscission , induction of dormancy , inhibition of seed germination and closure of stomata . However , none of these bioassay methods is specific and quantitative . Consequently , detection and estimation of ABA is done by physical methods only .

Occurrence of ABA

Abscisic acid occurs in various plant parts of mosses , ferns and a wide range of gymnosperms and angiosperms . However . it has not been reported to occur in any of the algae and liverworts . Instead , an inhibitor called lunularic acid has been extracted from several species of liverworts . ABA has been reported from the leaves of Ash (*Acer pseudoplatanus*) and birch(*Betula pubescens*) , tuber of potato and rhizome of *Pteridium* . Fruits constitute the richest source of ABA

Plants also contain substance closely similar to ABA in activity . These are phaseic acid from *Phaseolus multiflorus* , thiaspirone from tea leaves , 2- *trans* ABA from fruits of strawberry and (+) abscisyl β-D-glucopyranoside from lupin fruits . Plants subjected to wilting show a large accumulation of ABA . ABA is also shown to be an important component of β-complex inhibitors occurring in a number of species .

Chemistry of ABA

ABA is a sesquiterpene , a compound composed of three isoprene units. Since the gibberellins are also isoprenoids and like ABA are derived from Mevalonate , it appears that the early stages of the formation of these two hormones take place along the same biosynthetic paths . The synthesis of ABA from mevalonate added to some higher plant tissue has been demonstrated by (Phillips) . Some investigators believe that ABA may be a breakdown product of the photo- oxidation of xanthophylls such as violaxanthin ; similarity in molecular structure between the two types of compounds has given rise to this speculation .

Biosynthesis of ABA

There are two pathways by which can be synthesized in plants : (i) by oxidation of xanthophylls and (ii) from mevalonic acid (isoprenoid pathway).

It has been shown that violaxanthin , xanthophll , can undergo oxidation by enzymes forming xanthoxin . Xanthoxin is similar to ABA in several biological tests. It has been suggested that xanthoxin could be the immediate precursor of ABA for it can be converted into the latter. When tomato plants were fed with xanthoxin , it resulted in an increase in the ABA content due to its conversion to ABA .

The other possible pathway of ABA formation is from mevalonic acid . Mevalonic acid is also the precursor of gibberellins in plants . While gibberellinis a diterpene , ABA is a sesquiterpene . Therefore, some workers feel that the biosynthetic pathway of ABA is similar to that of gibberellins up to some extent .

Site of Synthesis

As ABA is closely related to carotenoids , it appears that chloroplast could be the site of ABA synthesis . Intact chloroplast isolated from fruit tissues of avocado was shown to synthesize ABA when supplied with mevalonate . Similarly isolated chloroplasts of bean leaves also synthesize ABA .

Transport of ABA

Transport of ABA has been studied in coleoptiles and stem sections . It is readily translocated systematically without being confined to any single direction . The pathway appears to be both xylem and phloem .

Metabolism of ABA

It is a common observation that plants inhibited by the application of ABA soon recover and resume their normal growth . Often ,a continuios supply of ABA is required to obtained a prolonged response . Therefore

it appears that plants can readily inactivate ABA . One of the possibilities is its binding to form abscisylglucoside . The second method by which ABA is rendered inactive in plants is by its oxidation to phaseic acid and dihydrophaseic acid . ABA is also made biologically inactive by its conversion to 2-trans ABA.

Physiological Roles of ABA

Bud Dormancy

In such plants it is the length of the day (photoperiod) which causes dormancy . Under the prevailing short days of autumn the duds enter the resting period and under long days these continue their growth . It is the long dark period of short days rather than the light period which is important. It is the mature leaves which are shown to perceive the effect of shot days . Now it is well established that the mature leaves synthesize the hormone ABA under short days . ABA is translocated through the phloem and reaches the apical bud where it causes dormancy.

Dormancy is terminated and bud resumes growth when the plant is exposed to A period of low temperature . This requirement of low temperature is met with in nature when the plant passes through the natural winter . Consequently , such plants resume growth in spring .

Environmental conditions which favor dormancy cause an accumulation of ABA and decrease in GA content . Therefore it is concluded that bud dormancy is under the joint control of two hormones , GA and ABA .

Seed Dormancy

Seeds of apple remain dormant and fail to germinate till these are exposed to a period of stratification . Such seeds show presence of ABA when dormant . When the seeds are stratified the ABA content falls and correspondingly the GA content increases . Here , dormancy is due to low GA and high ABA content .

Stem growth and Apical Dominance

Application of ABA causes inhibition of plant growth , the effect increasing with concentration . The stem , hypocotyl , root , coleoptiles , and leaves are all affected . Plants recover after a period of time or resume growth if the hormone is leached out .

Abscission

During ABA caused abscission , several derivative enzymes like protease , pectinase and cellulase increase . The middle lamella of the cell wall dissolves leading to abscission .

Senescence

Leaf disks of (*Tropaeolum majus*) floated on water soon become senescent. This is accompanied by rapid loss of chlorophyll , proteins and RNA . Acceleration of senescence by ABA is accompanied by decreased protein synthesis rather than increased protein degradation .

Environmental Stress and ABA

When wheat seedlings are subjected to leaf water deficit either by withholding water or by immersing the roots in carbowax , the leaves start wilting . Within a few minutes , the ABA content of the wilted leaves increases and is correlated with the degree of wilting .

Effect on Stomata

ABA has been reported decreased the stomatal aperture in plants. Stomatal closure is accompanied by loss of turgidity of guard cells which in turn is due to loss of osmotic potential . This may be through about by two methods : ABA might inhibit the formation of enzymes which in turn are responsible for the conversion of starch into sugar and formation of organic acids . The other possibility could be that ABA might change the permeability of guard cell membranes causing fall in the accumulation of organic ions leading to decrease in osmotic pressure of guard cells.

Mechanism of ABA Action

Growth inhibition caused by ABA is due to inhibition of both cell division and cell elongation . Such an inhibition , in turn , is mediated by regulating the activity of the nucleus . Abscisic acid has been shown to

inhibit the synthesis of both DNA and RNA . This might result in the inhibition of specific enzyme synthesis . At least , in the seeds of cereals it has been established that prior to visual emergence of seedlings hydrolytic enzymes such as α- amylase and protease increase . These enzymes are synthesized under the action of endogenous GA . Abscisic acid prevents the formation of these enzymes .

Ethylene

It was a common during the nineteenth century that gases present in smoke could modify plant growth. Neljubov (1901) was the first to show the importance of ethylene present in illuminating gas as a growth regulator of plants . The early work on ethylene was , however , not with respect to its effect on plant growth . It was mainly concerned with the practical application of smoke to induce flowering in pineapple and ripen bananas. The active component of smoke was shown to be ethylene by Crocker and Knight (1980) . Cousins (1910) suggested that plants might produce ethylene . An annual report by Cousins to the Jamaican Agricultural department mentioned that oranges should not be stored with bananas on ships , because some gases was formed from the oranges caused the bananas to ripen prematurity . this was the first suggestion that fruits release a gas that stimulates ripening. Crocker and his associates (1935) demonstrated that ethylene has a number of physiological effects on plants such as inhibition of stem growth , formation of adventitious roots on cuttings , epinasty of leaves , etc .

Occurrence and Distribution

Ethylene is produced by fungi , bacteria and higher plants . In higher plants more ethylene is produced in meristematic tissues and nodal regions . Usually the region of high concentration of auxin also corresponds to the region of high ethylene production in intact plants . The production of ethylene is also high in dormant buds of apple and the quantity decreases when the buds sprout . Its concentration is also high in leaves and flowers undergoing senescence . Ethylene formation also increases with the maturity and ripening of fruits .

Ethylene Antagonist

The response of plants to ethylene is reduced in the presence of carbon dioxide . Ethylene –caused inhibition of growth , leaf epinasty . abscission , fruit ripening , seed germination and bud sprouting are all shown to be reversed by carbon dioxide . Fruits stored

in an atmosphere of carbon dioxide remain fresh for a longer duration for the same reason . On the other hand removal of carbon dioxide by absorbing it in potassium hydroxide enhances ethylene activity . Also AgNO3 block ethylene production .

Factors affecting ethylene formation

1- Effect of Oxygen

Under anaerobic conditions , ethylene production is inhibited even when the tissue is supplied with methionine . Conversion of methionine to ethylene requires the supply of oxygen .

2- Effect of temperature

Optimum temperature required for ethylene formation varies from plant to plant . The optimum for apple is 30 C° .

3- Effect of stress

Stress created by herbicides , toxic substance , mechanical wounding and damage increases ethylene production .

4- Effect of Growth regulators

Auxin regulates ethylene production . Auxin increases ethylene formation .

Transport of Ethylene

Ethylene , being a gas can easily diffuse inside plant through the intercellular spaces . Ethylene can also dissolve in water and in lipophilic cell membranes . The movement of dissolved ethylene is passive and systemic . its movement dose not require metabolic energy and is not restricted to any single direction .

Physiological Roles of Ethylene

Dormancy of seeds

Application of ethylene is known to promote germination of seeds in barley and other cereals . This hormones also to be responsible for endogenous control of dormancy . The nondormant varieties of seeds produce more ethylene than the dormant varieties .

Root formation

Ethylene causes adventitious root formation on stem cuttings . In some plants ethylene treatment also promotes the formation of secondary roots and root hairs .

Epinasty

Ethylene also causes swelling of cells on the upper part of the petiole of leaf resulting in drooping of leaves (downward curvature).Epinasty is best exhibited by leaves of tomato , potato , pea and sunflower plants when exposed to ethylene .

Flowering

Ethylene inhibits flowering of plants almost universally . All phases of flowering namely , the initiation of primordial , development of flower bud and finally its opening are affected . Pineapple , other members of the family *Brmeliaceae* and *Plumbago* are exceptions . In these plants application of ethylene causes consistent and uniform flowering on vegetative plants.

Geotropism

When ethylene is applied to plants placed vertically upright , the main stem and the primary root bend at approximately right angles to vertical axis and grow horizontally.

Leaf abscission

Ethylene promotes leaf abscission . Role of ethylene in leaf abscission is probably because of :

1-Ethylene decreases auxin transport from the leaf blade to the base of the petiole where the abscission zone is located .

2- Ethylene treatment is also known to increase the activity of IAA oxidase which in turn reduces the auxin content .

3- Treatment with ethylene increases cellulase in the abscission zone and treatments which delay abscission also decrease this enzyme.

Fruit Ripening

During ripening of fruits a number of changes take place . Several degradative enzymes are synthesized . The cell membranes become broken down leading to disorganization of the compartmentation of the cell organells . Chlorophyll pigment is degraded leading to loss of green colour.

Ethylene affects plant growth:

They identified ethylene caused a triple response on pea seedling , it inhibits stem elongation , increases stem thicking , nd caused a horizontal growth habit , and inhibits leaf expansion .

Mechanism of Ethylene Action

The metabolic changes that occur during ripening are explained by two theories and in both ethylene could play a prominent role.

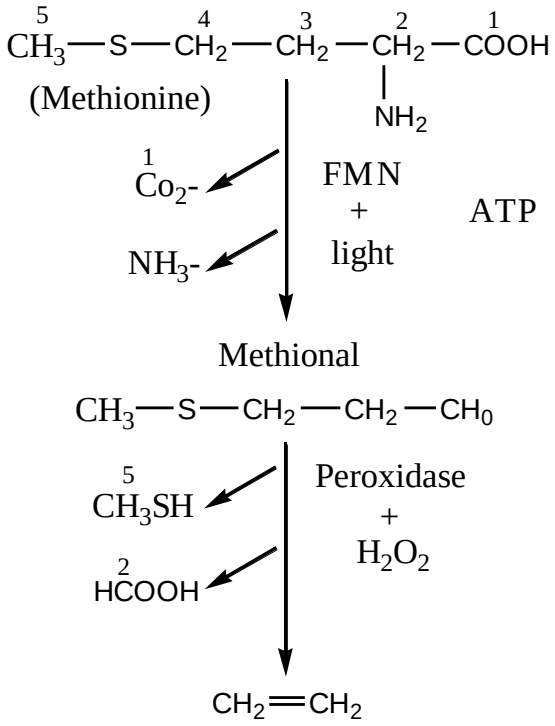
The first : is ethylene has been shown to cause an increase in tissue permeability .

The second : that new ripening enzymes are formed during the climacteric , and that the activity of these enzymes is in turn

the cause of the various metabolic changes that occur during and after the climacteric.

Ethylene Biosynthesis التخليق الحيوي للاثلين

Biosynthesis of $CH_2=CH_2$



ethylene الاثلين

The plant growth hormones

Introduction

It is now recognized that most if not all , of the physiological activity of the plant is regulated by a variety of chemical substance called *hormones* . The present of growth – regulating hormones in plants was first suggested by Julius von Sachs in the latter half of the nineteen century when he proposed that there were organ-forming substance in plants , which were produced in the leaves and translocated downward in the plant .

Charles Darwin 1880 suggested as did Seachs that plant grown might be under the control of special substances . He was able to demonstrate that the effects of light and gravity on the bending of both roots and shoots are mediated by the tip and that this influence can be transmitted to other parts of the plant .

Darwin for his experimental used canary grass (*Phalaris canariensis*) in its early sages of growth , he found if he exposed the tip of the coleoptile to a unilateral source of light , the coleoptiles would bend toward the light . And if he covered the tip of the coleoptile , so as to exclude all light from that area , the coleoptiles was insensitive to light and did not bend .

Boysen Jensen in the (1913) demonstrated that some substance , which originates in the tip , is responsible for the bending of unilaterally illuminated coleoptiles toward the light , he did not claim originally that this substance was a growth regulator .

Paal (1919) decapitated coleoptiles , replaced the tip asymmetrically and discovered that the coleoptile band away from the side with the tip . even in the dark . He suggested that there was a material substance emanating from the tip , which could stimulate growth of cells below the tip .

The next steps were o isolate this substance from the plant , Went (1928) placed freshly cut coleoptiles tip on small blocks of agar for a measured period of time and then placed the agar blocks asymmetrically on decapitated coleoptiles for 2 hours in the dark . The coleoptiles bend away similar that obtained when coleoptiles tips were placed asymmetrically on coleoptiles stumps . He then developed a method for determining the amount of active substance in the coleoptiles tips ; that is , he developed a bioassay for auxin .

Went found that the degree of curvature of the coleoptiles proportional within limits , to the amount of active substance in the agar blocks .

Kogl and Haagen-Smit isolated growth substance from human urine by application of the Avena test and the final product was given the name auxin A .

And they isolated by the same methods anther substance from corn germ oil very similar in structure and activity to auxin A and was given the name auxin B . Repeating the isolation from urine on larger scale and with the use of charcoal absorption method for removing the active substance , Kogl , Haagen-smit and Erxleben (1934) isolated the compound *heteroauxin* as is known today *indole-3-actic acid* .

Definitions

Since the discovery and chemical characterization of auxin , there has been an immense amount or research in the field of plant growth regulation . Needless to say , this prodigious amount of work brought forth a number of synthetic as well as natural compounds that were similar to IAA in their physiological activity . Also many compounds were discovered that counteracted the effect of growth regulators . A committee formed by the American Society of Plant Physiologists suggested the following definitions :

Plant regulators **are organic compounds other than nutrients , which in small amounts promote , inhibit , or otherwise modify any physiological process in plants .**

Plant hormones **(synonym : phytohormones) are regulators produced by plants , which in low concentrations regulate plant physiological roesses . Hormones usually move within the plant from a site of production to a site of action .**

Distribution of Auxin in the Plant

The highest concentrations of auxin are found in the growing tips of the plant , that is , in the tip of the coleoptile , in buds , and in the growing tips of leaves and roots . The concentration of auxin drops as one progresses from the tip to the base of the coleoptile , the highest content being found at the tip and the lowest at the base . Continuing from the base of the coleoptile along the root , there is a steady increase in auxin content until a high point is reached at the tip of the root . The concentration of auxin found at the tip of root is , however , nowhere near the concentration found at the tip of the coleoptile .

Thimann s found that auxin is present in the plant in two different forms , one that is easily extracted by diffusion methods and another

that is much more difficult to extract , necessitating the use of organic solvents . The easily extracted auxin is called free auxin and that which is hard to extract , bound auxin . It isnow generally accepted that bound auxin is the form that is active in growth and that free auxin is excess auxin in equilibrium with the bound auxin .

So far , it has been suggested that auxin in the plant is present in a free nonactive form and a bound active form , and that a dynamic equilibrium exists between the two .

Translocation of Auxin

Experiments by Darwin and by Boysen-Jensen demonstrating the movement of an active stimulus from the tip to the base of the coleoptile , led other investigators to assume that the movement of this stimulus was polar . This movement was thought to occur in a basipetal direction ; that is from tip to base .

Jacobs (1961) found that in *coleus* stem sections the ratio of basipetal to acropetal (base to tip) transport of auxin is 3:1 .

Also , some of the auxin produced by leaves is transported in the phloem tissues to other parts of the plant , a type of transport that is definitely not polar .

Goldsmith has clearly shown that auxin movement occurs acropetally as well as basipetally , although basipetal movement is strongly favored .

Velocities for auxin transport recorded in the literature vary with the type of plant being studied and the conditions under which experiments were performed . Thus , velocities anywhere from 6.4 mm \hour to 26 mm\hour have been observed .

Groups of investigators believes that a different in electrical potential between the tip and the base of the coleoptile controls auxin transport .

The base of the Avena coleoptile is more electropositive than the tip , the dark side of a unilaterally illuminated coleoptiles is more electropositive than the light side , and in a horizontally placed coleoptile the lower side is more electropositive . In each of these situations the translocation of auxin is toward the highest positive charge .

Gregory and Hancock (1955) have suggested that transport of auxin may be controlled to some extent by the metabolic of the cell ; that is , metabolic energy is involved . They found that a lack of oxygen inhibits the transport of auxin .

It is apparent from studies with Avena coleoptile sections that most auxin movement in the plant occurs in two different ways , one which is dependent upon metabolic energy and the other by simple diffusion . Basipetal movement in Avena sections occurs as a result of both diffusion and metabolic transport while acropetal movement relies only on passive diffusion .

The movement of auxin in the root system is also polar .

Physiological Effects

Auxin in some cases is stimulatory in others inhibitory and other cases is a necessary participant in the growth activity of another plant hormones (cytokinins and gibberellins).

We will discuss only the involvement of auxin in :

1-cell elongation
2-phototropism
3-geotropism
4-apcal dominance
5-root initiation
6-parthenocarpy
7-abscission
8-callus formation
9-respiration

Cell elongation

The action of auxin on cell elongation must involve some modification of the osmotic system of the cell .

The theories proposed from major studies on this problem have suggested that auxin may :

a-increase the osmotic content of the cell .
b-increase permeability of the cell to water .
c-cause a reduction in wall pressure .
d-cause an increase in wall synthesis .
e-induce the synthesis of specific RNA and protein (enzymes) which , in turn , lead to an increase in cell wall plasticity and extension .

Phototropism

When a growing plant is illuminated by a unilateral light by bending toward the light . The bending of the plant is caused by cells elongation on the shaded side at a much greater rate than cells on the illuminated side . This differential growth response of the plant to light , called phototropism , is caused by an unequal distribution of auxin , the higher concentration of the growth hormone being on the shaded side.

Many attempts have been made to explain why there is a higher concentration of auxin on the shaded side of a unilaterally illuminated coleoptile . This unequal distribution of auxin could be accomplished by

1-1light-induced inactivation of auxin ,

2- lateral transport of auxin ,

3- or inhibition of basipetal transport of auxin .

Geotropism

Like phototropism the geotropic response is controlled by an unequal distribution of auxin .

The stem as an organ which exhibits negative geotropism , the root as an organ which exhibits positive geotropism.

The accumulation of auxin on the lower side of a horizontally placed stem causes an accelerated growth to occur on the lower side causing the stem to curve upward . The horizontally placed root , whoever , will exhibit a positive geotropic response even though auxin concentrates on the lower side . Roots are much more sensitive to IAA than stems and the concentrations of IAA which stimulate cell elongation in stems are actually inhibitory to cell elongation in roots. The accumulation of auxin on the lower side of a horizontally placed root would , therefore retard cell elongation on that side . We should

also consider that the concentration of IAA in the upper side may be reduced to the stimulatory level for root cell elongation .

Apical dominance

The apical dominance might be because of auxin produced at the terminal bud and transported down word through the stem .

To explain why lateral bud grown should be inhibited by much smaller amount of auxin than is found in the apical bud , because the lateral buds are more sensitive than stems to auxin and that the concentration of auxin that stimulate stem grown is inhibitory to lateral bud growth .

Root initiation

It has been found that the action of auxin in root is similar to that in stems , but the concentration of auxin stimulatory to stem growth are inhibitory to root growth , roots are much more sensitive to auxin than stems , high concentration of IAA to roots retard root elongation and noticeable increase in the number of branch roots .

Application of IAA at the end of young stem stimulates the rate of formation and number of roots initiated .

Respiration

Jmes Bonner recognized in 1933 that auxin has a stimulatory effect on respiration . His work led him to suggest that auxin avtivly only takes place in the presence of oxidative metabolism .

Auxin stimulates respiration and that there is a correlation between increased growth due to auxin treatment and increase in respiration.

Callus formation

The auxin activity stimulated cell elongation in the plant , it also is active in cell division . IAA not only causes a proliferation of cell , but also under some condition may cause a dedifferentiation of these cells, that is , cause the formation of adventitious roots .

Parthenocarpy

Fruit development in the absence of pollination does .The development of fruit in this manner is called parthenocarpic development , and the fruit that is formed is called parthenocarpic fruit.

Auxin play an important role in the development of fruit by pollination growth of the pollen tube and fertilization all contribute to the gush of auxin responsible for fruit development .

The controlling influence of natural auxins on the abscission of leaves was first suspected in1933 when Laibach(1933) showed that a substance contained in the extract of the orchid is capable of preventing abscission . Support of this observation was given by LaRue who demonstrated the delaying effects of various synthetic auxins on the abscission of *Coleus* leaves . Since that time , a great deal of confirmatory work has been performed , clearly establishing indole -30 acetic acid (IAA) as an important controlling factor in the abscission of plant organs.

Distraction of IAA

There are three methods by which IAA molecule becomes inactivated :
1-oxidation by light (photo-oxidation)

2-oxidation by enzymes

3-binding of auxin molecules to other organic forming inactive complexes.

GIBBERELLINS

Discovery

The discovery of gibberellins dates back to 1920 when Kurosawa , Japanese pathologist , while investigating the *bakanae* disease of rice plants , observed that the infected plants excessively elongated and become spindly . It was later shown that the disease was due to the infection of rice plants by the fungus , *Gibberella fujikuroi* (Fusarium moniliformae) . The fungus could be isolated and grown in culture on synthetic medium . The culture medium was freed from the fungus by filtering and the filtrate was applied to rice plants . This also caused the usual symptoms of bakanae disease characterized by excessive elongation , as if the plant was infected with the fungus . This leads to an important conclusion that the fungus secrets some substance into culture filtrate when grown in the medium or in the plant which it infects . This substance is responsible for causing unusual stem elongation . the active principle was named gibberellin after the name of the fungus *Gibberella fujikuroi* (Yabuta and Sumiki , 1938).This substance was later purified and crystallized and active franction was named gibberellic acid (Curtis and Cross , 1945; Stodola *et al* .,1955) . Although the initial discovery of gibberellin was in the fungus , now it is known that it acts as a hormone and is produced by the flowering plants independently without the help of the fungus . A number of new gibberellins are shown to covered in quick succession . Now all the known gibberellins are shown to be produced of plant metabolism , some in the fungus and the rest in higher plants .

Extraction and Purification

Diffusion Technique

This technique is similar to the one already described for auxin . It was employed for the first time to extract gibberellins from apical bud of sunflower plants*(Helianthus annus*) . The plant organ is excised and the cut end is plugged into an agar block . High humidity and continuous light are maintained . Substances present in the plant part are now allowed to diffuse into agar for 12-24 hours . From the agar block

gibberellins is extracted with solvents and then tested with a suitable bioassay method . This method is useful to study the rate of biosynthesis of gibberellins in a given plant material over a period of time . It is also being employed to pinpoint the site GA biosynthesis in plants .

Solvent Extraction

Gibberellins have been extracted by solvents both from *Gibberella fujikuroi* and also from higher plants . the procedure for its extraction is more or less similar regardless of the starting material . The plant material is extracted in methanol or acetone or with mixture of one of these solvents with water . The organic solvent is evaporated under pressure . The resultant aqueous fraction is acidified . to pH 2.5 and extracted with ethyl acetate and then with sodium bicarbonate . The combined extract is evaporated under reduced pressure . This gives a mixture of gibberellins along-with impurities . From this crude extract , the presence of a particular giberellin can be detected by gas chromatography .

Occurrence and Distribution

Gibberellins have been shown to occur universally in bacteria , fungus , algae , mosses , ferns , gymnosperms and angiosperms . Both monocots and dicots produced this hormone. So far 55 different gibberellins are known to occur in plants as native plant hormones . However , no single plant is reported to contain all of them . Many plants contain more than one gibberellins at the same time .

Gibberellins occur in all the parts of a plant body . Developing seeds are reported to contain higher quantity of gibberellins than vegetative parts . As a result , immature seeds *Phaseolus coccineus* , , wild cucumber , castor , *Pharbitis nil* and other plants are frequently used to isolate gibberellins .

In addition to free gibberellins , plants also contain gibberellin glucoside . While the former are acidic, the latter are neutral in nature . During the maturation of the seed , free gibberellins decrease with a simultaneous increase in gibberellin glucoside which appear to be inactive storage forms . When the seed germinates , these glucosides are hydrolysed releasing free gibberellins .

Plants also contain kaurene , kaurenal and kaurenoic acid which show gibberellin-like activity . These occur as intermediates in the biosynthetic pathway of gibberellins. Consequently , their biological activity is due to their ultimate conversion .

Chemistry of Gibberellins

Gibberellins consist of the gibbane carbon skeleton and possess biological properties (1)

(1) **Gibbane Skeleton**

similar to gibberllic acid . However , plants also contain substance whose chemical nature is not established but all the same possess biological properties similar to known gibberellins .

Chemically , gibberellins are diterpenoids consisting of two terpen units . Each terpene unit consists of ten carbon atoms and is in turn made up of two isoprene units . An isoprene unit has a 5-carbon structure(2)

(2) **Isoprene unit**

The different gibberellins have the following structural characteristics:

1- All gibberellins possess a gibbane carbon skeleton .

2- Gibberellins are acidic in nature . However , the number and location of carboxylic group (COOH) varies from one gibberellin to the other. All gibberellins having 19 carbon atoms possess a single COOH group. This carboxylic group is always found in position 7 . On the other hand the 20-carbon gibberellins have one more or COOH groups present in 4, 7 and 10 positions .

3- The number and position of hydroxyl group (OH) also differs . Some gibberellins have a single OH group while in the others it is absent . Whenever it is present , it is either in position 3 or 13 . Whereas in gibberellins found exclusively in the fungus , the OH group is in position 3 , in gibberellins from higher plants it is in position 13 .

4- Ring A is unsaturated and the degree of unsaturation differs from one gibberellin to the other .

Bioassay of Gibberellins

1- Dwarf Maize Test

The single gene mutants of maize (*Zea mays*) remain dwarf as the stem fails to elongate due to deficiency of gibberellins . External application of GA causes rapid stem and leaf sheath elongation .

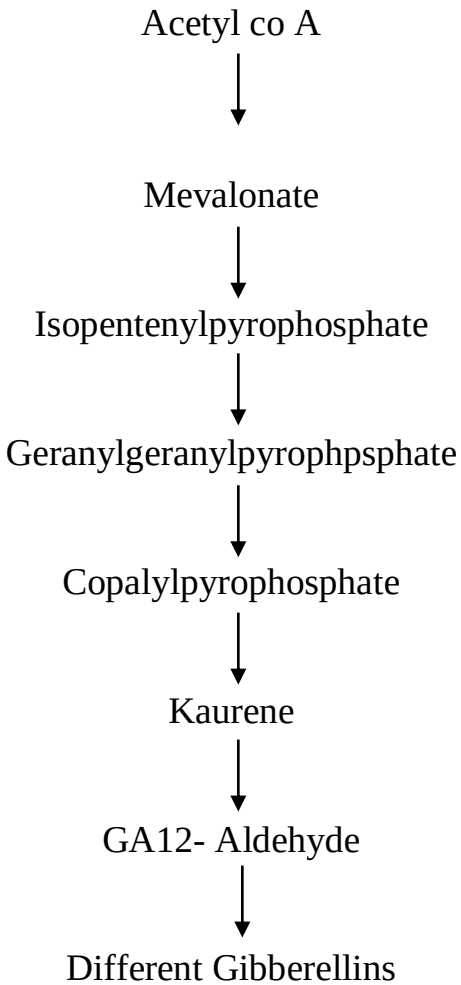
2- Barley Endosperm Test

Gibberellin causes synthesis of α -amylase in the aleurone cells and this enzyme

in turn is responsible for the conversion of starch in the endosperm into reducing sugars .

Biosynthesis of Gibberellins

Acetic acid is the starting material for the biosynthesis of gibberellins . Three molecules of this are linked together to form a molecule of mevalonic acid . Mevalonic acid in turn is converted into anther straight chain compound isopentenyl pyrophosphate . This is a 5-carbon compound . Four molecules of this undergo condensation to form a diterpene called geranylgeranylpyrophosphate . This is a 20-carbon compound and it undergoes cyclization (closing up of rings) giving rise to kaurene . This reaction is carried out by the enzyme kaurene synthetase in two steps . Kaurene undergoes stepwise oxidation giving rise to kaurenol , kaurenal and kaurenoic acid . Kaurenoic acid undergoes a series of reactions and finally gibberellin results .



(3) Biosynthesis pathway for gibberellins

Site of synthesis

Gibberellins are synthesis in the shoot tip , root tip and in the developing seeds . In the shoot tip , it is the leaf and the bud primordial which are responsible . Roots tips are also shown to synthesize GA and supply the same to above ground parts .

Transport of Gibberellins

Transport of gibberellins unlike that auxin , is passive and systemic. Gibberellin moves readily in all directions in all tissues including phloem and xylem .They are synthesis by young leaves , roots and immature seeds embryo.

Mccomb reported that GA moves in the same pattern as the carbohydrate translocation system and with a similar velocity.

The Physiological Effects of Gibberellins

Seed Dormancy

Seeds of many species although , do not germinate even under favorable conditions of moisture , temperature and in the present of air or oxygen. These are said to be dormant and the process is called dormancy. Dormancy has the ecological advantage of restricting germination to only certain parts of the year when the environmental conditions are congenial for the survival and establishment of the seedling.

Dormancy may be due to the presence of hard seed coat which dose not permit gaseous exchange and entry of water. In some species the seeds are dormant as the embryo is not fully developed at the time of shedding . Seeds may also be dormant because of the presence of germination inhibitors in the embryo or the seed coat , which impede germination . In some cases seeds remain dormant till these are exposed to light , dark or a period of low temperature. Some types of seed dormancy can be overcome by the application of GA .

The dormancy of these seeds is traced to the presence of germination inhibitor identified as abscisic acid (ABA) . Such dormant seeds are also deficient endogenous germination promoters , particulary the gibberellins .

When the dormant seeds are subjected to low temperatures the ABA content decreases or the GA content increases.

Bud Dormancy

In potato , the buds present on the tubers do not sprout soon after harvest as these undergo a period of dormancy . However , when GA is applied , the buds sprout soon after the tubers are harvested and sometimes even while these are still attached to the parent plant .

In nature the tubers remain dormant for a few weeks after harvest . During this period the level of endogenous gibberellins in the tubers increase . Further some new gibberellins which were not present earlier appear. Therefore gibberellins act as endogenous regulator of bud dormancy in potato tubers as well .

Seed Germination

Gibberellin has been shown to play an important role in the germination of cereal seeds such as barley, wheat and others . present in the endosperm of the seed nourish the growing embryo . How ever , these food substance are in the insoluble form , namely , as starch and protein . Before these can be used by the embryo these have to be rendered soluble by their hydrolysis into glucose and amino acids. This is being carried out by - amylase and protease respectively.

Stem Growth

By far most dramatic effect of gibberellins on plant is their effect on stem elongation . Plant treated with GA grow taller .

Gibberellin-caused elongation has the following characteristic features :

1- Enhanced stem growth is not due to increase formation of nodes and internodes but results from rapid elongation of internodes that would have formed even without GA application. Therefore , GA –treated

plants do not differ from control plants in the number of nodes and internodes.

2- Elongation if internodes is due to both cell division and cell elongation.

3- Younger internodes respond better than older ones .

4- Plants grown in light respond better to GA than those grown in the dark.

Genetic Dwarfs

In maize several dwarf mutants are known .These have resulted by the mutation of a single gene controlling stem growth . Such plants remain short in stature . When gibberellin is applied the dwarf plants respond readily and show marked stem elongation and become comparable in height to corresponding tall varieties . Here the degree of stem elongation is made use of as a bioassay method for gibberellin.

Dwarf maize mutants do not show normal stem growth because of the total absence of endogenous gibberellins , which may be due to a genetic block in its biosynthesis pathway by mutation .

Rosette Plants

In some plants like *Hyoscyamus* and *Samolus* the stem remains short and condensed and the leaves are crowded together as the internodes fail to elongate. This gives A rosette appearance . The stem is in the rosette condition and the plants are in the vegetative phase when these are grown under short photoperiods . However when these plants are exposed to long days rapid stem elongation takes place , which is referred to as bolting , which is accompanied by flowering .

Some plants like radish , carrot and beetroot remain in the rosette form when the temperature is high . Bolting and flowering take place only after exposing the plants to low temperature (vernalization)

Root Growth

External application of GA dose not promote root growth .

Gibberellins inhibit the formation of adventitious roots on the stem cuttings of most plants .

Sex Expression

Application of GA increase the number of male flowers and decreased the number of female ones .

Parthenocarpy

The native gibberellins and gibberellins-like substance play a major role in the development of fruit under conditions . The young developing seeds contain relatively high amounts of gibberellins, that the gibberellins produced during seed development is translocated out into the fruit tissues where it exerts some control over fruit development.

Gibberellins have been found very reliable in producing parthenocarpic fruit-set and in many cases show higher activity than the native auxin in this resoect.

Mechanism of Gibberellin Action

One of the most important effects of GA is the elongation of cells of the intact stem . There are several hypotheses to explain the mechanism of GA in this process.

Increase in the Auxin content

Application of GA has also been shown to reduce IAA oxidase activity .

Increase in Osmotic Pressure og Cells

Gibberelin has been shown to cause synthesis of amylase in barley aleurone cells . This enzyme converts starch to reducing sugars . Consequently osmotic pressure of cells increase .

Nucleic Acid Synthesis

GA results in the stimulation of RNA and protein synthesis .

Lipid Metabolism and Membrane Permeability

GA –induced enhancement of nucleic acids is considered to be an after-effect of growth . Even formation of amylase is a much later event . Therefore the primary action of GA is at some site other than nucleic acid and protein synthesis . This site appears to be the membrane permeability .

Cytokinins

Discovery

The effect of auxins and gibberellins is mainly due to their effects on cell elongation . A plant cannot indefinitely grow exclusively by cell elongation because the extent to which individual cells can be stretched is limited . Therefore cell division which contributes to an increase in the number of cells is an important process of plant growth . The process of cell division is also controlled by hormones . The presence of cell division causing substance was suspected in the developing fruits and seeds which show active cell division , mainly confined to the embryonic part of the seed . The embryo has been shown to receive the necessary cell division causing substances from the or coconut milk is rich in cell division causing factors . Coconut milk not only causes cell division and growth of coconut embryo but also of excised and artificially cultured embryos of *Detura* and other plants . Later extracts of developing fruits of maize , Ginkgo , Aesculus and other plants were also shown to contain cell division promoting substance .

Coconut milk is a complex substance . Therefore , attempts were made by Steward and Shantz (1952) to isolate and purify the active principle present in it. Two substance , namely , diphenyl urea and myo-inositol, were isolated . However these substance by themselves could not duplicate the effect of coconut milk, the reason being that coconut milk contains a variety of substance such as auxins , gibberellins , amino acids , nucleic acids and so on . The cell division causing effect of coconut milk could not be ascribed to any single substance . Therefore , the effect appears to be a combined effect of all the substances .

A real breakthrough in the discovery of cytokinins came from the work on tissue culture studies . Folke Skoog and his associates in the University of Wisconsin attempted to grow pieces of pith tissue of a tobacco plant in test-tube by supplying them with major and minor elements , vitamins and sucrose . The pith tissue could not grow . When auxin was added to the medium , cells showed elongation but failed to divide and multiply .

A year later , a cell division causing substance was extracted as a degradation product . of DNA by autoclaving (heating at high temperature and pressure) herring sperm DNA (Miller *et al* ., 1955). This substance could causing cell division in tobacco pith cells , it was called " kinetin" . Chemically , kinetin was to be 6-furfurylaminopurine . However it was soon discovered that kinetin dose not occur in any of the plants but it was only an artifact or degradation product of DNA molecule.

As mentioned earlier , cells of tobacco pith when excised and cultured in artificial medium do not grow . The cells elongate but do not divide when auxin is added to the culture medium . However the cells start dividing profusely and form a parenchymatous mass of unorganized cells when kinetin is added along with auxin . Such a mass of cells known as callus . Kinetin alone is not effective . Therefore , it become soon established that cells require at least two growth factors for their division , namely , auxin and kinetin . These two growth substances interact and bring cell division .

Adenine , the purine base found in nucleic acids , was also found to be similar to kinetin in causing cell division although it is much less active.

Kinetin , benzyladenine and the other known cytokinins are synthetic chemicals which do not occur in plants . Therefore , a search was made for the naturally occurring cell division causing factors in plants . The first source was extracted , purified and crystallized from immature maize kernels (letham, 1963) . It was named zeatin after the name of the plant *Zea mays* . Chemically , zeatin is a substituted adenine . Its occurrence was soon established in other plant species as well . Zeatin was also chemically synthesized .

By definition , cytokinins are plant hormones which cause cell division in tobacco pith tissue when used along with auxin .

Extraction and Purification

Cytokinin is extracted from the plant material with 80 percent ethanol . The tissue is ground and repeatedly extracted with the solvent for a few hours . The extract is centrifuged and is evaporated under vacuum and the dry

residue is dissolved in water . The crude extract can be directly used to estimate cytokinin by any one of the bioassay methods . The impurities present in the extract can be removed by chromatography . Either Whatman chromatographic paper or thin layer can be used .

Occurrence and Distribution

Cytokinin occur in plants both freely and also as components of transfer RNA specific for the amino acids , serine and tyrosine . Although zeatin was originally extracted from maize , it has also been isolated from the root exudates of sunflower , leaves of begonia and culture filtrate of the fungus . In addition to zeatin , plants also contain zeatin riboside .An analogue of zeatin , namely N6-isopentenyl adenine (IPA), is present in transfer RNA of yeast , and also in pea , cron and other plants.

Cytokinins occur widely in higher plants . In general , embryo and endosperm of developing seeds , apical meristems , root nodules and all such regions , which show active cell division show the presence of cytokinin. The content decreases in older non-meristematic tissue.

Structural Requirements

All the cytokinins so far known , are derivatives of adenine . These differ from one by the nature of side chain . The attachment of side chain has be on the N6 position of adenine molecule . Substitution at positions other than this either reduces the activity or completely renders the molecule inactive . Even without any side chain , adenine molecule itself has a weak cytokinin activity . Attachment of a side chain increases the activity of the molecule . The activity is further enhanced if the side chain has one or more double bonds . The side chain may contain a ring as in benzyl adenine or a straight chain as in zeatin . The synthetic cytokinins may have furfuryl , benzyl , butyl , phenyl or methyl side chain , while all naturally occurring cytokinins contain isopentenyl side chain . Therefore , adenine moiety of the molecule is an essential requirement for activity .

Bioassay of Cytokinins

Tobacco pith bioassay

Cytokinin causes cell division in tobacco pith and soybean cotyledon callus tissue grown aseptically on synthetic medium . Consequently the fresh and dry weight of callus tissue increases .

Radish Cotyledon Bioassay

Isolated cotyledon of seeds of radish (*Raphanus sativus*) show very little expansion when floated on water . However , when cytokinin is size . Cotyledon expansion is proportional to logarithm concentration.

Xanthium Leaf Disk Bioassay

Excised leaves or leaf disks floated on water soon become yellow owing to loss of chlorophyll . This is accompanied by degradation of RNA and proteins as well . This phenomenon is referred to as senescence . The senescence is delayed and the leaf remains green for a longer duration with the addition of cytokinin .

Transport of Cytokinins

Unlike auxins and Gibberelins , Cytokinins are poorly translocated in the living tissue of plants. This was shown by applying C14 labelled benzyl adenine to bean leaves. Droplets of this cytokinin applied to the leaf do not appear to move but remain localized . However , cytokininis carried passively along the transpiration stream in the xylem from the root , which is the main source of its production , to the aerial parts of the plant body . Consequently , xylem sap of many plants shows high concentration of this hormone .

Physiological Role of Cytokinins

Dormancy

Lettuce seeds require the presence of light for germination in addition to moisture , air and suitable temperature . It is the red part (660 nm) of white light which is the most effective . However , the seeds can be made to germinate in the dark by applying kinetin. That is, the red light requirement for germination can be replaced by cytokinin. Seeds of tobacco , white clover and others also behave similarly.

The effect of red light and kinetin is synergistic . It means that the individual effects of both is less than their combined effects.

Kineten also overcome seed dormancy of *Striga* and other root parasites .In nature , seeds of *Striga* do not germinate till these come in contact with the root system of the host plant . The exudates of the host root probably supplies the chemical signal in the form of cytokinin which in turn causes the germination of parasitic seed.

Cell Division

The most important effect of cytokinins is stimulation of cell division in excised tissues artificially cultured *in vitro* . However , the present of auxin is also necessary . In tobacco pith callus , auxin alone added by itself to the culture medium although causes more enlargement of cells it dose not promote cell division. On the other hand , cytokinin added alone also dose not promote cell division. However, when both auxin and cytokinin are added together , cells rapidly divide and the callus tissue grows. Therefore , the present of both the hormones is necessary for cell division. Cytokinin is thought to increase thr synthesis of DNA and mRNA , and auxin the ribosomal RNA component of cell division.

Cell Enlargement

Cytokinin causes enlargement of cells of leaf disks taken from fully expanded leaves . Similar expansion has also been reported in the excised cotyledons of radish seeds floated on cytokinin solution . Cytokinin appears to be essential for leaf growth . If the root tip . which is an active site of cytokinin biosynthesis , is cut off , the cotyledons and leaves fail to expand. However , exogenous application of this hormone replaces the requirement of root tip for leaf expansion . In intact plants , application of cytokinin inhibits longitudinal growth of stems and roots but increases the diameter of these organs.

Cytokininn also substitutes for light requirement for leaf expansion , i.e. the leaves of dark- grown plants are narrow and folded . However , these expand and unfold when exposed to light . Instead of exposing to light , application of cytokinin also brings about similar effect.

Morphogenesis

Root and Bud Differentiation

The initiation of roots and buds is controlled by the interaction of auxin and cytokinin. The ratio of these two hormones is the determining factor.

The cells of tobacco pith do not either grow or differentiate when auxin or cytokinin is added alone to the medium . However , when the medium contains both auxin and cytokinin in the ratio of 10:1, pith cells grow forming a mass of unorganized cells(callus) . However , the cels do not organize and differentiate into tissues and organs. If the ratio of auxin to cytokinin is increased by either decreasing the concentration of cytokinin or increasing the concentration of auxin , a number of roots initiated from the callus . If the ratio of auxin to cytikininis increased , a number of shoot buds are initiated . Therefore , it is the quantitative balance and interaction of auxin and cytokinin which controls differentiation of roots and buds.

Development of Plastids

Cells of tobacco pith callus fail to form chloroplasts either in light or in dark . In light , although plastids are formed these do not become differentiated into normal chloroplasts till cytokine is added to the culture medium . In the dark , with the addition of cytokinin , proplastids without grana are formed . However , these become differentiated into normal chloroplasts on exposure to light . Therefore , it appears that in tobacco callus cells chloroplasts are differentiated by the interaction of cytokinin and light .

Development of Fruits and Seeds

After fertilization the ovary develops into fruit . During the early stages of fruit development , growth of the ovary is due to active cell division and the later development is mainly due to cell enlargement . Growth of the ovary caused by cell division , at least , appears to be controlled by the endogenous supply of cytokinins , for its level is shown to be quite high at a time when the developing fruit is showing maximum cell division as in cotton , apple , banana and others . Further excised fruit tissue fail to show cell division unless cytokinin is also added in the culture medium .

The early growth of the embryo in the developing seed also appears to be controlled by the cytokinin . Such embryos if removed from the ovules and cultured on synthetic media , do not grow unless a cytokinin or cytokinin containing substance like coconut milk or yeast extract are added to the culture medium . Therefore , cytokinins play an important role in the development of fruits and seeds.

Delay of Leaf Senescence

When a leaf becomes old it becomes senescent and finally falls or dries up on the plant. When the leaf becomes senescent the content of chlorophyll , proteins and RNA rapidly decreases . This may because of two reasons . Either the rate of synthesis of these substances becomes reduced or the degradation may become enhanced .

Apical Dominance

Application of cytokinin reduces apical dominance . The lateral buds of intact plants which , otherwise remain arrested , can be made to grow by applying kinetin . Inhibition caused by externally applied auxin to decapitated stem is also reversed by the cytokine , i. e. the effect of cytokinin is antagonistic to that of auxin in apical dominance. Here the cytokinin appears to favor the differentiation of vasculature of lateral buds , which becomes connected with that of the main stem . This facilitates the flow of the water and nutrients from the stem to lateral buds . As a consequence , these emerge out as branches.