CHAPTER

10

Phytohormones

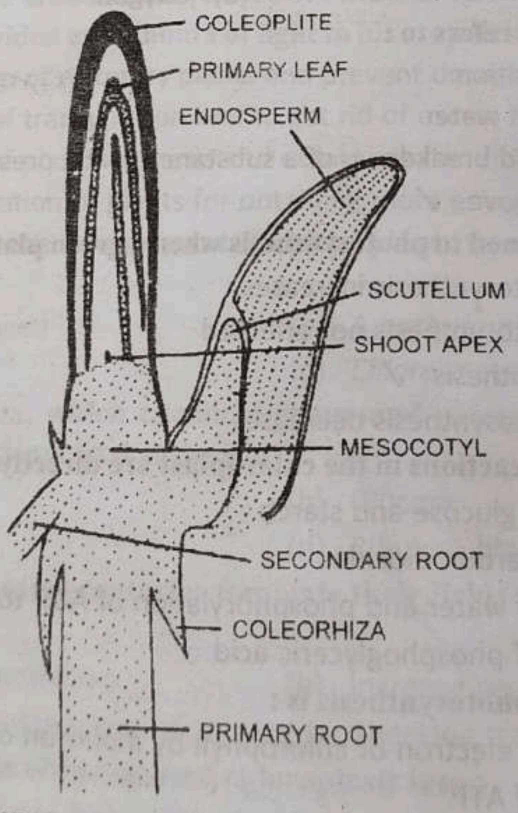
10.1 INTRODUCTION

Phytohormones Or Plant Hormones

The plant growth may be regulated by several factors such as light temperature and growth regulating substances.

Phytohormone is an organic substance produced naturally in plants controlling growth and other functions at a site remote from its place of production and active in minute amounts.

As a result of experiments it has been observed that plants tissues have some chemical substances which have a profound influence on the growth and development of plants. It has also observed that even the small amounts of these substance are effective. These substances are quite different from food material and enzymes. Plants physiologist called these substance variously as **hormones, growth hormones, phytohormones.**



OAT (AVENA SATIVA COLEOPLITE)

Fig. 1. Oat (*Avena sativa* coleoptile).

The hormone (Excitant-activator) can be defined as organic compound produced in one tissue of plants as a result of metabolism and migrating to other

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tissue where in minute quantity it affect growth. The best known plant hormones are the auxins.

The term hormone was first used by Starling (1906) to refer to substances secreted in living beings and capable of evoking response in other regions.

Although auxins, phytohormones, growth regulator, etc. are commonly used for growth hormones. A brief terminology of these substances is as follows :

(1) Growth regulators : These control the plant growth.

(2) Flowering regulators : These control the flowering.

(3) Plant hormones (Phytohormones) : These in low concentrations regulate the physiology process.

(4) Growth hormones : These regulate plant growth. Hormone Concept

Most of our knowledge about plant hormones has been derived from experimental work on the seeding of grasses, especially *Avena sativa* (oat) : During germination of oat-a hollow cylindrical structure with a conical tip-develops and enclose the shoot apex and first foliage leaf which ultimately breaks through it. The structure is called **coleoptile.**

Experiments have shown that when the *Avena* coleoptile tips are exposed to light coming from one side, coleoptile bends towards the direction of light (Darwin 1880). If a coleoptile tip is discapitated and separated by a cut made several m.m. below the apex the growth rate is retarded immediately Bapsen Jensen (1913). If the tip is again replaced, resumption of growth takes place. RE. Went (1928) collected this hormone in an agar blocks and termed auxin.

10.2 DEVELOPMENT ROLES OF PHYTOHORMONES

A. THE AUXINS

Discovery : The existence of auxins was realised as early as in 1S&7, when Charles Darwin and his son Francis Darwin demonstrated that canary grass (*Phalaris canariensis*) coleoptiles did not show phototropic response when the tip was either removed or covered with a foil cap. Between 1910 and 1930 investigations conducted by various scientists demonstrated that the agent which perceived light stimulus in a phototropic response was of chemical nature. The first successful isolation of the chemical was carried on in 1926 by a Dutch botanist, F.W. Went. He found that oat coleoptile tips contained a chemical which was involved in phototropic curvature of the coleoptile; The chemical was also involved in endogenous control of growth rates. The chemical which was called auxin (from the Greek auxin meaning to grow), by Kogl, Hagensmit and Went, was later on isolated from a variety of materials including human urine (Kogl and Kostermann, 1934) malt and corn meal (Hagensmit et. al, 1942). Now we know that auxins are present in the tissues of all higher plants, although only ift small quantities. They are of fundamental importance in the physiology of growth and differentiation.

Chemical Structure

The first crystalline auxin was obtained from human urine by Kogl and others in 1934. Chemically it was shown to be indole 3-acetic acid (abbreviated IAA);

Since then it has been found that IAA occurs as a principal auxin in most plant species. Here are many other compounds closely related chemically to IAA, which also possess auxin like properties.

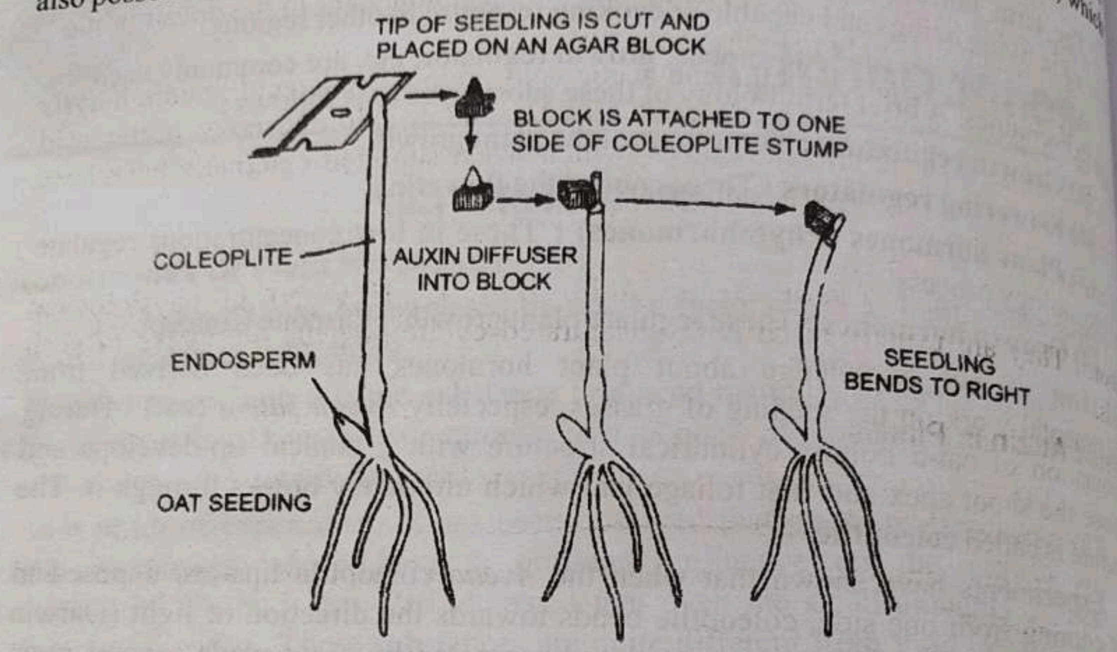


Fig. 2. Diagrammatic representation of the effect of auxins on plant growth.

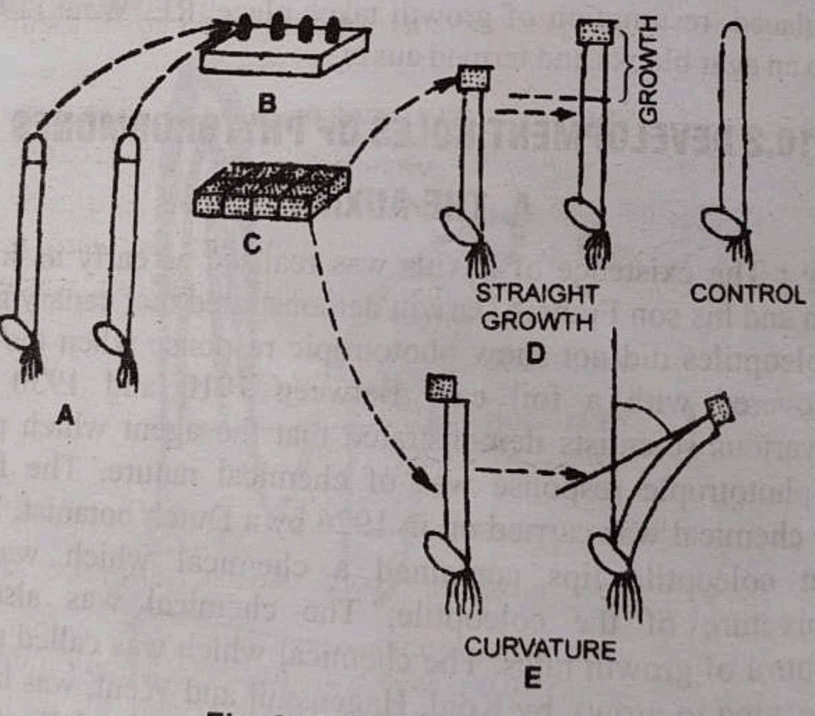


Fig. 3. *Avena coleoptile.*

Auxins are organic compound characterized by its capacity in low concentration (below 10^{-3} M or 0.001 M) to induce elongation in shoot cells and inhibition of elongation of root cells.

The auxins occur in very small quantities and are of wide distribution. They may be isolated from the plants material and has been grouped as Auxin '*a*' ($C_{13}H_{12}O_5$). Auxin '*b*' ($C_{18}A_{30}O_5$) and heteroauxin or indole-3-acetic acid ($C_{13}H_8O_2N$).

Auxin are found present in the class of plants and in form of free auxins, precursors and fixed auxins. The auxins are naturally present in the plant cells and are synthesised in the metabolic process. Usually auxin; Synthesised in one tissue and translocated in other organs. The auxins are most abundant in the apical portion and less abundant in lower portion.

Phynel acetic, alpha naphthalene acetic acid, indole acetic acid, indole butyric acid, 2, 4-dichlorophenoxy acetic acid (2-4-D) and trichlorophsnoxy acetic acid (9-4-ST) are same synthetic substances, which when supplied externally have been reported to possess the properties of auxins.

Chemistry of Auxins

Auxins resemble Indole Acetic Acid (IAA, $C_{10}H_9O_2N$) in physiological actions. They are usually acids with unsaturated cyclic nucleus derivative of such acids.

Role of Auxin in Plants

(1) Auxins promote growth in stem by promoting cell elongation.

(2) Auxins inhibit the growth of root.

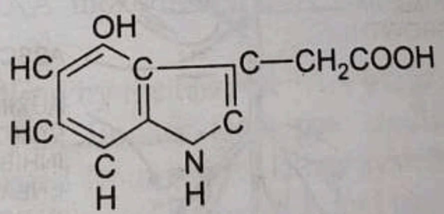
(3) Auxins causes the divisions of cells in adventitious roots, limits and cambium.

(4) Alpha naphthalene acetic acid prevented their normal elongation and resulted in the formation of dwarf shoot.

(5) Alpha napthyl acetamide prevents longing and causes them of grow stiff woody and erect.

(6) The application of indole butyric acid is used to faciliate the development of roots in cuttings of plants during vegetative reproduction.

(7) Alpha-naphthalene acetic acid and Malic hydrazine inhibit the sprouting of potatoes and are used in U.S.A., Holland and Germany in the treatment of stored potatoes. This save much valuable food from waste.



(8) Alpha chlorophenoxy propionic acid causes inhibition or flowering in vegetable drops.

(9) Auxins may prove a great weapon war, because when highly concentrated solution of these auxins sprayed on enemy land by air or some other means, they produce a good devastation of crop plants and form of basis of bilogical warfare.

(10) The auxins also play an important role in movement of plants.

(11) Application of auxin prevents the abscission of leaf fall.

(12) Application of auxins also help in the production of seedless fruits (parthenocarpy).

(13) The spray of auxins increase number of female flowers in cucurbits.

Auxin influence on growth process is as follows

Auxin is responsible for initiating as well as promoting cell division. Cal development is certainly related to auxin action. Proliferation of parenchyma cells is also associated into auxins. In tissue culture the cell division is entire dependent on the presence of auxin. Seasonal activity of cambium in plants has been observed to be closely parallel by a similar variation in auxin synthesis by developing shortening of internodes is also due to auxin. IB A has been observed root initiating growth regulator. The polar transport of auxin is very clear pronounced in cuttings.

In many plants only the apical bud grows and the lower axillary buds are suppressed removal of the apical bud, however, results promptly in the growth one or several at the lower buds. The auxin of the terminal bud is thus responsible for inhibiting, the development of lateral buds by aphenomerion known as apical dominance.

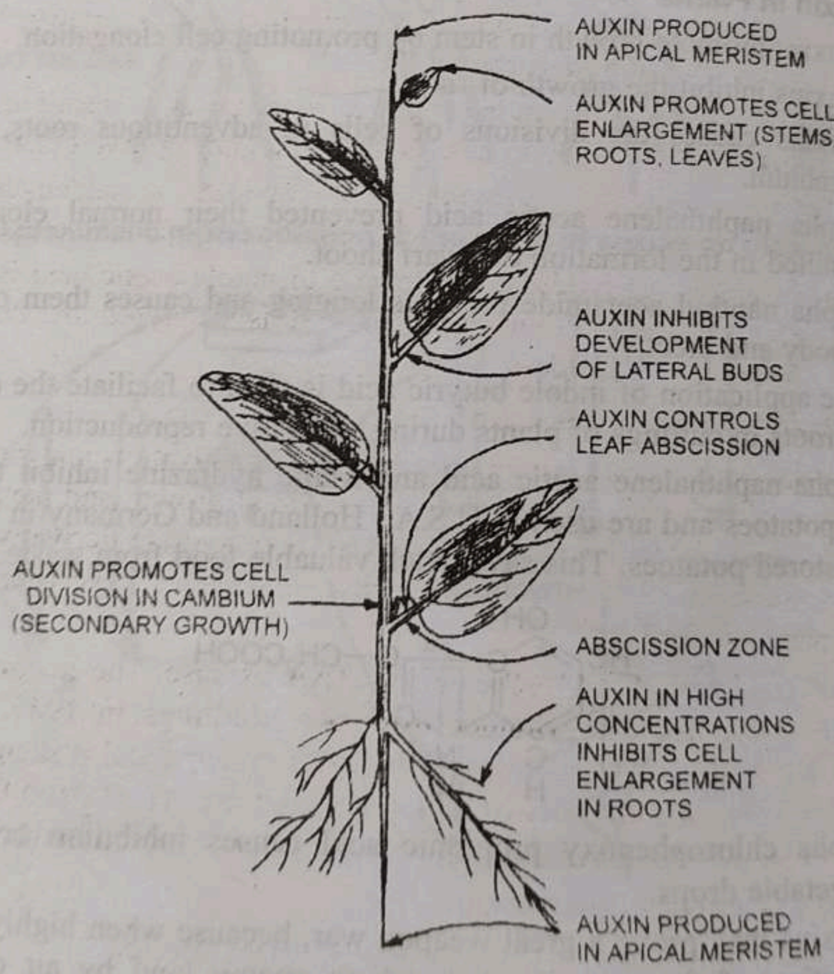


Fig. 3. Diagrammatic representation of various effects of auxins of plants.

The formation of abscission layer int he premautre fall of leaves, flowers, and fruits believed to be due to dissolution of the middle lamella. Some auxins (IAA, NAA, 2, 4-D, etc.) have been used successfully in checking this.

Auxin generally inhibits flowering but promote flowering in Pineapple (Anannas). Use of 2, 4-D in eradiction of weeds is well known.

Auxin Biosynthesis

Indole acetic acid, the principal naturally occurring auxin, is synthesized from the aromatic amino acid tryptophan. Enzyme system, capable of converting tryptophan to IAA, has been isolated from spinach leaves and oat coleoptiles. IAA can also be synthesized from tryptomine and indole pyruvic acid by leaf discs, or crude leaf extracts from pine apple leaves. It has been suggested that tryptophan is first converted to indole acetaldehyde either through indole pyruvic acid or through tryptomine. Indole acetaldehyde is then converted to indole acetic, acid. The reactions involved are :

(1) Conversion of tryptophan to indole pyruvje acid is anoxidatvie transmination reaction and is catalysed by the enzyme tryptophan transminase. Ammonia of tryptophan is accepted by a suitable keto acid, which in turn is converted to an amino acid.

(2) Conversion of indole pyruvic acid to indole acetaldehyde involves decarboxylation (of side chain) reaction and is catalysed by the enzyme indole pyruvate decarboxylase.

(3) Conversion of tryptophan to tryptomine is a decarboxylation (of side chain) reaction and is catalysed by the enzyme tryptophan decarboxylase.

(4) Conversion of tryptomine to indole acetaldehyde is oxidative determination process catalysed by the enzyme amine oxidase.

(5) Oxidation of indole acetaldehyde to indole acetic acid is the final step in IAA synthesis from tryptophan. It is catalysed by the enzyme indole acetaldehyde oxidase.

Auxin Inactivation Degradation

Auxin inactivation and degradation : Inactivation and degradation of IAA plays an important role in the regulation of plant growth and metabolism. IAA may be inactivated by binding with protiens or carbohydrate derivatives IAA glucoside, IAA arabinose and IAA inositol-arabinose glycosides have been [discovered from several plant tissues.]

IAA can be oxidised by the enzyme IAA oxidase. The enzyme was first isolated by Y.W. Tang and J.D. Bonner from pea seedlings in 1947. Subsequently, the enzyme has been isolated from several other systems and it seems to be ubiquitous. The enzyme has the property of peroxidase and uses H_2O_2 and O_2 for the oxidation of IAA to 3-methylene oxindole. Carbon dioxide is evolved in the process.

Answer the following :

(i) Name the principal auxin in most plant species.

(ii) Which kind of stem/coleoptile segment is more responsive to external auxins etiolated or green?

(iii) What is the usual concentration range of auxins in which they cause stem elongation :

$$10\ M, \quad 10^{-2}\ M, \quad 10^{-4}\ M, \quad 10^{-6}\ M$$

(iv) Which plant hormone is generally considered to be a rooting hormone?

(v) Which ion is believed to be extruded from cytoplasm in auxin induced cell wall growth?

(vi) Which one of the following may act as a second messenger for auxin action : IAA, Polyamines, Metallothioneins, D.N.A.?

(vii) Name the enzyme involved in the conversion of indole acetic acid to 3-methylene oxindole.

[**Answer :** (i) Indole acetic acid, (ii) Etiolated, . (iii) 10^{-6} M, (iv) Auxins, (v) H^+, (vi) Polyamines, (vii) IAA oxidase]

B. GIBBERELLINS

Gibberellins is another group of plant hormones discovered recently. Work of Japanese scientists led to the discovery of Gibberellins Y in 19th century. The Japanese farmers found that called it Bakanae or foolish seedling disease. For this disease fungus *Gibberella juikuroi* was responsible.

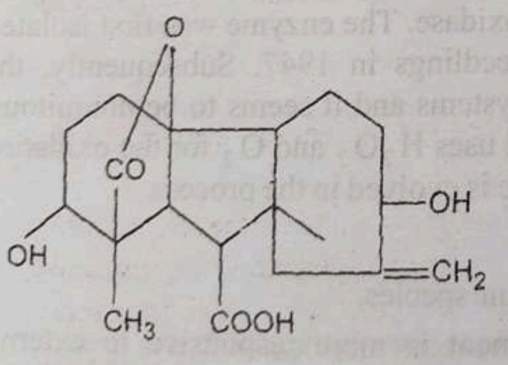
Kurosawa (1926) found that the fungus in its culture growth give out a substance which has some effect on growth as fungus itself. In. 1938 Yabuta and Sumiki finally succeeded in isolating a per crystalline chemical and named it as Gibberellin. In 1950 Gibberllin remain of great interest among scientists.

Six Gibberellins were isolated by Cross et al., (1961) from fungus *Gibberella juikuroi* McMillin (1961) also isolated three Gibberellins from bean seeds. At present there are yearly 32 new stains of Gibberellins. Chemically these compounds are all of the tetracyclic type of ring compounds.

Main effect of the Gibberellins is in promoting the elongation of the internodes of the shoots,. Besides this Gibberellins are also effective in promoting the expansion of leaf inhibition of lateral buds, increase in diameter of the steam and initiation of flowering, etc.

Corcoran and Phinney 1962, told that Gibberellins are involved in seed maturation process. Gibberellin treatment can induce parthenocarpic development of seedless fruits.

Chemically the gibberellins are different forms of gibberellins acid containing gibbonic skeleton. Structure of some common gibberellins is as follows :



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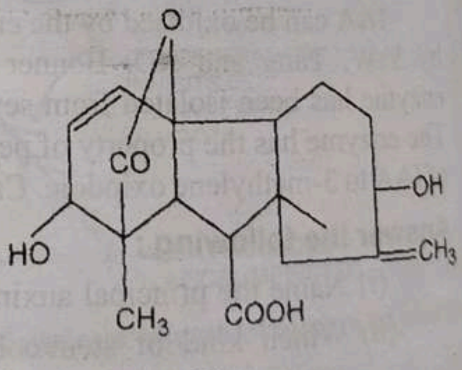


GA2

Physiological effect of gibberellins are :

(1) Stem elongation : The internodes increase in length.

(2) Promotior of following in long days plants.

(3) Dwarf plants : They convert genetically dwarf plants into plants of normal height. Addition of GA to cabbage plant converts the head or dwarf stem into stem which is 608 feet tall. Rosette plant of sugar beet is an extreme case of dwarfism.

Lang (1956) .showed that such a stem can undergo rapid growth if it is raised with gibberellin.

(4) Parthenocarpic fruits : They cause parthenocarpy in tomatoes, apples and pears. It has improved fruit in tomatoes.

(5) Breaking of Dormancy : These have been observed effective in breaking the dormancy in potato tubers and in tree buds.

Mechanism of Action

Gibberellins seem to affect plant growth and development in a variety of ways. In many cases, it has been found that the GA stimulation of plant growth in dwarf seedlings is associated with increase in auxin content. Further, GA suppresses the activity of indole acetic acid oxidase, the enzyme responsible for IAA degradation. Base donhese observations, it was suggested that GA affects plant process through auxins. However, several effects of gibberellins are independent of auxins, the auxins, the auxins can not replace gibberellins. For example, application of GA to barely aleurone layers causes a rapid increase in a-amylase activity, white auxins do not have such effect.

It appears that most of physiological effect of GA are mediated via increased enzyme synthesis and membrane permeability. These aspects are described in the following paragraphs.

[I] Enzyme Induction

Several plant enzymes such as α- and β-amylases, proteases, ribonuclease, RuBP carboxylase, β-1,3-glucanase and nitrate reductase, etc. are known to be induced by gibberellins. J.E. Vainer, G.R. Chandra and MJ. Chrispeels in 1960's demonstrated that GA caused *de novo* synthesis of hydrolytic enzymes during seed germination. GA seems to increase both transcription as well as translation, as the increase in enzyme activity is inhibited by actinomycin D (an inhibitor of transcription) and cycloheximide and fluorophenyl alanine (inhibitors of translation).

During GA treatment, more of radioactive phenylalanine was incorporated into proteins, indicating an increased translation. There is also an increased incorporation of ribonncleotides into RNA during GA treatment.

[II] Membrane Permeability

Some effects of gibberellins seem to be mediated via increased permeability the cellular and organellar membranes.

Gibberellins may affect the permeability of plant membranes either by affecting the synthesis of molecules involved in membranes or by changing their organization. The enzymes choline transferase and pbosphatyl choline glyceride transferase increase b2 to folds after 2 to 12 h GA treatment (Johnson and Kende, 1971). These enzymes are involved in the synthesis of lecithin, a major phospholipid membrane component. Further, GAS treatment increases the incoiporation of ^{14}C Choline in (partially purified endoplasmic reticulum preparations).

[III] Gibberellin Interaction with DNA

Gibberellins and possibly auxins and cytokinins as well, have been reported to interact with DNA and change is physical properties. GAS, IAA and kinetin change the melting points of DNA. GA has been shown to bind with adenirie-thymine rich segments of DNA *in vitro* conditions (Kessler and Snir, 1969). However, it is not known whether such interactions of GA with DNA are necessary for various physiological and biochemical responses.

[IV] Analogy with Animal Hormones

Structural similarities of GA with steriod hormones, (of animal origin) have prompted suggestions that GA may have a mode of action that resembles that of steroid hormones of animals. Accordingly, the hormone may bind to a specific site (receptor) in the membrane of the target cell (the cell where the hormone is to elicit response). There is a structural change in receptor protein as a result of this binding which then moves on to the nucleus, hinds to the chromatin DNA and activates transcription.

Bio synthesis of Gibberellins

Biosynthesis : Biosynthesis of gibberellins has been examined in immature seeds. However, there are evidences in favour of gibberellin synthesis, in roots as well. Most of the biosynthetic steps take place inside the palstids. By using tracer technique (use of radioactive isotopes) it has been shown that acetate is the primary precursor of gibberellin synthesis. The synthesis of various gibberellins from acetiate may be considered to involve four groups of reactions :

(1) Synthesis of linear chains of geranyl pyrophosphate from acetate.

(2) The cyclization to form Kaurene.

(3) A series of oxidation steps, including contraction of 'B' ring to form the presumed first synthesised 20-carbon gibberellin, GA_{12} and the 19-carbonGA_4.

(4) Inter conversions of various GAs.

Commercial Use of Gibberellins.

Commercial uses of Gibberellins : GAs have found extensive use in horticulture and food industry. They are often on vienyards to increase the number and size of grapes in the cluster. GA_3 has been also used to produced 'Thompson's seedless' grapes. Improvement in the size, colour and quality of apples, pears and many other fruits is also achieved by spray gibberellins. In these cases, the hormone stimulates bud formation and fruit set.

Gibberellins are also used to increase α-amylase activity in germinating barley seeds which is used for malt production in beer industry.

One of the most notable uses of gibberellins is their application to sugar cane crop. They promote the elongation of cane internodes without decreasing the sugar content. This application is particularly important to Indian sub continent, where sugar cane is an important cash crop and foreign exchange earner.

Answer the following :

(i) Name the disease of rice plant, with which the discovery of gibberellins is associated,

(ii) Dwarf pea seedlings are often used in the bioassay of plant hormone : auxin, gibberellins cytokining, abscissic acid,

(iii) Which one of the plant hormones, (auxin, GA, cytokinin an abscissic acid) is often considered as a flowering hormone.

(iv) Name one enzyme of aleurone layers which is induced in the presence of GA.

(v) Gibberellins not only induced the synthesis of α-amylase, but also activate its release from barley aleurone "layers". True or false.

(vi) Which cell organelle is primarily involved in the biosynthesis of gibberellins?

(vii) Name the two carbon molecule, which is primary precursor of gibberellin biosynthesis.

(viii) Which plant hormone is often used in promoting the growth7 of sugar cane internodes?

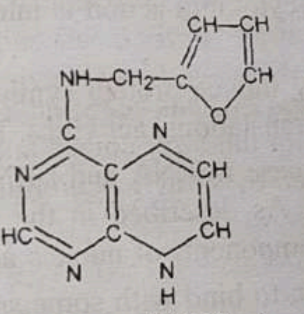
[**Answers :** (i) Bakanae, (ii) Gibberellins, (iii) GA, (iv) α-amylase, (v) True, (vi) Plastids, (vii) Acectate, (viii) Gibberellins]

C. CYTOKININS

The discovery of cytokinins dates back to 1921 when Austrian plants physiologist, G. Haberlandt discovered vascular tissues of plant containing unknown difrusable factor, which stimulated cell division.

Skoog and Jablonski (1954) discovered cytokinins during culturing of tobacco tissue. They noted that a specific cell division factor was contributed by a vascular tissue. A Miller (1956) crystallized and identified his factor as 6 **furfuryl amino purine.** This substance was found to be specific on cytokinesis, hence names as kinetin. **Lethani** (1963) proposed the term cytokinin for kinetin and analogous substances. Zeatin is another type of kinetic found in maize embryos. Cytokinins now used for the substances which can stimulate cell division.

Chemically the cytokinins are mainly 6 substituted purine derivatives.



KINETIN

Cytokinins stimulate cell division, stimulate cell enlargement, interacts with auxins *i.e.*, the control of Morphogenesis. Cytokinins are helpful in shoot elongation and growth, stimulate root initiation and increases root Cambial activity. They are helpful in breaking dormancy of seeds and promote germination. They delay the senescence and also control several other physiological activities. Physiological roles of cytokinins are as follows :

1. Cell Division : It is capable of causing cell division and DNA and RNA synthesis. It possess a 'true cell division' factor.

2. Cell enlargement : Cytokinin cause enlargement of cortical cells of tobacco roots upto four times their normal size.

3. Morphogenesis : It may cause organs formation in a variety of tissue culture, *e.g.*, formation of plastids from protoplastids, differentiation of trachieds through action of lignin biosynthesis, induction of flowering and induction of parthenocarpy.

4. Counteraction of apical dominance.

5. Breaking dormance : These have been tested in breaking the dormancy of seeds and some other plant organs. This not only break the dormancy but also promote the germination of seeds.

6. Delay of senescence : In detached leave of Xanthium, Lang (1957) has proved this fact.

Mechanism of Action

The most important aspect of cytokinin action is increased mitosis, (cell division) which involves increased synthesis of nucleic acids and proteins. However some other physiological effects are not easily explained through such a mechanism. Some possible mechanisms of cytokinin action are as follows :

[I] Increased nucleic acid and protein synthesis

All maturity occurring cytokinins are purine derivatives. This chemical structure relationship between cytokinins are nucleic acids has prompted researchers to suggest that cytokinin action in some way is exerted through influencing and protein metabolism. The nucleic acid metabolism can be influenced in a variety of ways. Some cytokinins are known to intercalate with DNA. Such an interaction will modify the template action of DNA and hence is replication and transcription.

Increase in nucleic acid and protein content during cytokinin treatment has been reported in several systems. In developing rice grains and in leaves of tobacco, kinetine causes marked increased DNA, RNA and protein. This stimulation of activities in leaf discs, has been suggested to be a factor in the deferral of senescenece by kinetin. This cytokinin action is inhibited by the inhibitors of RNA and protein synthesis.

Cytokinin treatment may induce protein synthesis by increasing one or more aspects of transcriptional and translational activities. These are :

(1) Cytokinins may increase m-RNA and t-RNA content, which are involved in protein synthesis. As described in the earlier paragraphys, cytokinins increase nearly all components of nucleic acids in many systems.

(2) Cytokinins-are known to bind with some specific ribosomal proteins. This may increase the translational efficiency of ribosomes.

(3) Cytokinin isopentenyl adenosine or its hydroxylate derivative, zeatin, is located as a modified nucleoside, adjacent (3-end) to anticodon in plant t-RNA. In this form, they may affect the covalent linking of charged t-RNA to m-RNA during protein synthesis. In fact, F. Fittler and R.H. Hall (1966) have observed that the presence of a cytokinin adjacent to anticodon in seryl t-RNA from yeast, is essential for protein synthesis in that system.

[II] Increased Enzyme Activity

Several enzymes are known to increase during cytokinin treatment. These crugnnes include, those catalysing a variety of ... of diverse nature.

a-amylase, some photosynthetic enzymes and some enzymes of thiamine synthesis. An increased enzyme activity may be the result of increased nucleic acid protein synthesis, as discussed in the earlier section.

[III] Involvement of receptor protein and a second messenger

Besides, specific protein binding-sites in ribosomes, a protein from tobacco and soybean nuclei has also been isolated, which mediates the kinetin stimulation of RNA synthesis. It is proposed that such a protein serves as the^site of cytokinin action and increased DNA, RNA synthesis is due to the action of this hormone protein complex.

The binding with a receptor protein may also evoke the release or activation of a second messenger molecule. As with other plant hormiones, the second messengers may be Ca^{2+} calcium binding protein calmodulin or polyamines. The second messenger in turn may evoke other biochemical responses.

Biosynthesis

Cytokinins

Cytokinins are synthesized in the roots from where they are transported to the shots via xylem tissues. Biosynthesis of purine type of cytokinins is more clearly known than of other types. Various components of a purine are derived from several small molecules including formyl tetrahydro Mate, aspartate, CO_2, glutamin, glycine and methylidyne tetrahydrofolate. The biosynthesis of purine nucleotide (purine base + ribose sugar + phosphate) begins with the ribose phosphate and purine ring is built on it, step by step. The first nucleotide to be synthesized by this process is inosine monophosphate (IMP). IMP is then converted to either guanosine monophosphate (GMP) or adenosine monophosphate (AMP).

The five carbon side chain attached either to the purine base or to purine nucleotides, in most cytokinins is derived from isopentenyl pyrophosphate. Isopentenyl pyrophosphate is derived from mevalonate utilizing the usual pathway of isoprenoid or steroid biosynthesis; It is believed that the adenosine nucleotide (AMP) is incorporated into t-RNA first and then it is modified; aside ‘chain is added. Using tobacco tissue cultures, C. Chen and R.H. Hall (1969) have demonstrated that mevalonate is incorporated into dimethylallyl and then there is an enzyme system capable of introducing dimethylallyl side chain into t-RNA. Alternatively, processed nucleotides (as cytokinins) are synthesized first and then incorporated into t-RNA.

Answer the following

(i) Which plant hormone is primarily involved in inducing cell division?

(ii) What kind of nucleic acid molecule is believed to contain a cytokinin molecule as one of its component nucleotides (DNA, m-RNA, t-RNA, r-RNA)?

(iii) Which is the most important plant hormone causing the expansion of cotyledons?

(iv) Who for the first time proposed that roots produced some hormones which delayed the senescence of leaves?

(v) Which enzyme catalyses the oxidative degradation of cytokinins?

(vi) Increase in nitrate reductase activity by cytokinin seems to be mediated via another plant hormone. Which hormone is that?

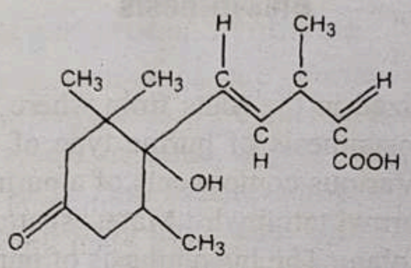
(vii) Local application of cytokinin to the leaf delays its senescence. This is believed to be due to increased rate of respiration by the leaf section treated with the cytokinin.

[**Answers :** (i) Cytokinin, (ii) t-RNA, (iii) Cytokinin, (iv) A.C., (v) Chibnall, (v) Cytokinin oxidase, (vi) Ethylene, (vii) False]

C. ABA (ABSCISSIC ACID OR TERPLINIC ACID)

Abscissic acid is growth inhibitor substance which causes dormancy of seeds or buds and sometimes named as dormin. It was first isolated in crystalline form by W.C. Lice and H.R. Cairns in 1957. The principal components of inhibitor B described by T.A. Bennet Clock and N. P. Kefford (1953) was also AbA.

Chemically it is $C_{15}H_{20}O_4$ acid, soluble in organic solvents.



ABSCISSIC ACID

Structure and Distribution

Abscissic acid is a sesquiterpene consisting of 15-C atoms. It is unique among plant hormones in having an asymmetric carbon atom. It has a six carbon ring structure to which a side chain is attached. Because of the asymmetric carbon atom (carbon-1) it occurs in two enantiomorphic forms, R-abscissic acid and S-abscissic acid. The naturally occurring form is S-abscissic acid. This form of AbA is dextrorotatory (+). The degree of optical rotation of + ABA is perhaps the highest among known biological molecules. Because of this property, ABA is often detected by optical rotation dispersion technique. The AbA is often detected by optical rotation dispersion technique. The AbA has been synthesized in laboratory also and synthesized AbA is generally a racemic (±) mixture.

AbA is cannon in plant materials, ranging in concentration between 0.03 to 4.0 mg/kg fresh weight. On molar basis the concentration ranges between 0.001 and 1 um. It is of general occurrence in monocots, dicots, gymnosperms and some ferns. Generally fruits and seeds contains the highest amount of AbA. However, it is easily transportable to other tissues as well. In fruit itself, it comes from its site of synthesis, the mature leaves. Most likely the translocation takes place in the phloem and xylem.

AbA has been isolated from ferns, gymnosperms and angisoperms, when it accumulates in appreciable quantities. It has also been found that the fungus *Cercopora rosicola* synthesizes and secretes large amounts of AbA in the culture medium. AbA is apparently not present in liverworts. But another compound-lunularic acid seems to play a role similar to AbA in liverworts.

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Physiological Effects

[I] Inhibition of seed germination

Exogenous supply of AbA inhibits germination of most non-dormant seeds. For causing an inhibition, AbA must be continuously present. As soon as washing of the seeds, the germination can take place. Endogenous AbA also inhibits germination. The AbA content of dormant seeds is high and it decrease during germination.

[II] inhibition of seedling growth

AbA inhibits growth of the seedlings in some cases. In *Glycine max* for example, 1 mM AbA inhibits seedling growth by about 50% in 48 h. In some cases, similar growth inhibition is observed in almost 2 h. Auxin stimulated elongated growth in coleoptile is also inhibited by AbA. It has been suggested that inhibits seedling growth by decreasing the water potential.

[III] Inhibition of bud growth

Exogenous AbA induces but dormancy in woody plants. As in dormant seeds AbA content in dormant buds is high and it decreases by treatments which lead to the breaking of dormancy. The hormone inhibits lateral growth of the bud, as has been reported in tomato. Plants in which bud growth is inhibited by far-red treatment, also have high AbA content. Further, cultivars with high apical dominance contain high AbA levels. It appears that this high level of AbA is maintained by the IAA content of the tissues, as IAA has been invariably implicated in apical dominance.

[IV] Stomatal Closing

The most significant and best known effect of AbA is its control of stomatal closing. Exogenous application of AbA to epidermal strips causes stomatal closure. In fact, factors such as water stress, chilling, etc. which induce stomatal closure, also increase AbA content of guard cells. The response of AbA on stomatal closing is very fast and occurs within a few minutes of AbA application.

[V] Geotropism

There seems to be some involvement of AbA and perhaps other inhibitors as well in geotropism. Appreciable amounts of AbA have been detected in maize root tips. The accumulation of AbA in the tip appears to require light and gravity. AbA produced in the cap seems to be translocated basipetally and to stimulate a positive geotropic response. Further, the exogenously applied AbA induces positive geotropism although it inhibits root growth.

[VI] Senescence and Abscission

Numerous studies indicate that AbA is an endogenous factor involved in senescene and abscision of leaves and' other plant organs. Exogenous application of AbA induces primary yellowing in leaf tissues in a variety of species ranging from deciduous trees to herbaceous plants such as *Tropaeolum*. It also accelerates abscission in leaves in a number of speçies. In many studies, it has been found that a non-volatile substance is released during senescence of leaves, which accelerates abscission. DJ. Osborne has called this substance as *senescence factor* (S.F.). Many scientists have suggested that this senescence factor is AbA.

Exogenous application of AbA promotes loss of chlorophyll, protein and RNA and promotes symptoms of senescence in detached leaves, although relatively higher concentration (1 to 5 mM) of AbA are required. Further, AA content increases during senescence of detached leaves. For example in detached tobacco leaves the AbA content increases by about 6 folds in light and 22 folds in dark in 7 days. Further, the application of kinetin which inhibits senescence prevents the rise in AbA content. However, the effects of AbA on attached leaves are not clear cut.

Role of Abscissic Acid in Drought Resistance

AbA seems to play an important role in plant during water stress and during drought conditions. Following observations suggest such a role :

(1) AbA promotes stomatal closing and thus reduces water loss due to transpiration.

(2) AbA content of mesophytic plants increases when the plant is subjected to water stress and the content decreases when the plant is no longer under tress.

(3) In many cases physiological effects of water deficit are similar to those of AbA treatments.

D. ETHYLENE

Discovery : The history of the discovery of ethylene as a planthormone, starts with 19th century when plant growes noticed the effect of smoke and naming gases on plants.

The first recorded effect can be credited to the Russian botanist Dimitry N. Neljobow (1901) working in St. Petersburg, who demonstrated that ethylene affects C plant growth. He also established that ethylene was emanated from illuminating gases and it had three types of effects on pea seedlings-stem elongation, stem thickening and horizontal growth. Many, other plants physiologists also demonstrated the effect of illuminating gases on plants. In 1912, A.F. Sievers and R.H. True demonstrated that the combustion gases from the kerosene stove were beneficial in the ripening of lemons. W.C. Crocker and L. I. Knight (1908, 1913) identified ethylene as the active constituent of both, illuminating gas and tobacco smoke E.M. Harvey (1913) determined that biological responses produced from paper could be attributed to ethylene. In 1942, F.E. Denny confirmed that ethylene was active substance in kerosene fumes causing the ripening of lemons.

The first report that plant materials evolve ethylene came from H.H. Cousins acid in 1910, who observed that when oranges and banana were stored together during shipping, sortie gas was emanated from oranges which caused ripening of banana. In 1934, R. Gane provided chemical proof that ethylene was produced by ripe apples.

Physiological Effects

1. Seed germination : Ethylene is known to break dormancy and induce germination of lettuce, ground nut, wheat, clover, and cocklebur seeds. It also causes that increased extension growth of the seedlings in cocklebur. The extension is maximum at 0.3 ppm ethylene. The maximum germination however, is obtained at about 40-50 ppm ethylene. On the other hand, inhibition of germination by ethylene has been recorded in maize, *Potentilla* and some weeds.

2. Growth inhibition and morphogenetic effects : In most cases exogenous atiwt of ethylene inhibits plant growth. In most dicots the elongation growth of the roots and leaves is inhibited. But the hormone enhances radial growth, as a result both stem and root swell in response to ethylene. Auxin and ethylene when applied exogenously or produced in partially inhibitory concentrations, act synergistically to inhibit root elongation and swelling of the root tips (G. Bertell, E. Bolander and L. Eliasson 1990).

3. Epinastic responses : Exposure to ethylene causes epinastic movement in petioles as a result the leaves bend down. This is because of more growth on upper or lower side of the petiole. Epinasty is also caused when excess of auxin is applied. This is believed to be due to endogenous ethylene production. Stem epinasty is also caused due to ethylene, as in tomato.

4. Flowering inhibition and sex expression : Ethylene inhibits flowering in most plants, although it is known to promote flowering in mango and pineapple. In Plumbago indica also, a short day plant, flowering can be induced by ethylene in long days. Flowering induction in pineapple with response to certain auxins has been to endogenous ethylene production.

5. Ripening of fruits : Acceleration of fruit ripening was the first discovered effect of ethylene. The hormone is now known to accelerate ripening of mature fruits in mm cases including banana, apple, tomato, avocado, etc. In apple fruits, the softening of fruits increases with the increase in ethylene concentration, reaching maximum at 50 ppm ethylene. In most fruits, this softening is brought about by chemical changes in the cell wall materials.

Ethylene appears to play an important role in natural ripening of fruits. During ripening of most fruits, the ethylene production increases, sometimes by as much as 50 to 1000 folds. The ripening process of fruits can be delayed by removing ethylene from the vicinity of developing fruits or by maintaining low oxygen levels around fruits.

6. Acceleration of senescence and abscission : Ethylene is an important hormone governing the senescence and abscission of plant parts, both natural induced. It accelerates senescence of leaves, flowers and fruits. Endogenous ethylene increases during senescence. Supply of ethylene degrades chlorophyll. Amino-cyclopropane carboxylic acid (ACC), a precursor in ethylene biosynthesis, also accelerates chlorophyll degradation in detached leaves.

Abscission is the most widely demonstrated response of ethylene. It induces abscission of leaves, fruits, petals and flowers. The threshold concentration to induce abscission is generally 1 ppm, although in some cases as low as 0.01 ppm ethylene (as in fox glove corola) can induce it. The abscission, increases with ethylene concentration, saturating at about 10 ppm. Exposing fruiting cotton plants to 0.5 ppm ethylene causes 100% abscission of young fruits and floral buds within 2 days. In this respect, it is believed that the initial effect of ethylene is to lower the auxin content. Ethylene controls various physiological processes through following activities :

(i) **Ethylene eject on enzymes :** There are reports which show that ethylene has a direct effect, on enzyme activity. According to Nelson (1939) activity of trypsin is induced by ethylene.

(ii) **Eject on membranes :** Ripening fruits show many changes in their permeability and retention of soluble components which compell to believe that ethylene brings a change in permeability which in turn softness and increases respiration but recent findings confirm that a change in membrane properties is an effect of ripening rather than a cause. There are many reports in avocado, banana, bean potato and pea which show that ethylene has no effect on membrane permeability. On the other hand, Guttenberg and Beythien (1951) in Rhoeo leaves observed that ethylene increased the rate of deplasmolysis but this was criticised by Burg (1968).

(iii) **Enzyme induction :** It is believed that ethylene induces many enzymes because ethylene treated tissues have been formed to contain higher a concentrations of certain enzymes (protease, inveftase, etc.) than the control.

(iv) **RNA synthesis :** Ethylene has been reported to increase RNA synthesis in various processes—such as in epinasty of tomato leaves (Turkova *et al.* 1965), in abscission (Abeles 1973) and in preclimacteric fruit (Cherry 1977).

(v) **Chromatin activity :** Recently Holm *et al*, (1970) reported that ethylene retards the growth of apical part but increases the elongating and basal region of the stem in seedlings of soya bean. This was accompanied by decrease in RNA levels and chromating activity in apical zone whereas increase in elongating and basal regions.

(vi) **DNA-metabolism :** Depending on the tissue, ethylene may promote or inhibit the growth or even may not have any effect on growth. According to Burg *et al.*, (1971) ethylene regulates DNA synthesis through some action on structure microtubules which is required in formation of spindle during mitosis.

Ethylene releasing chemicals : Ethephon (2-chloroethylphosphonic acid) BOH (2-hydroxyethyl hydrazine) ethylprophylphosphate and monoethylsulphate release by ethylene.

Biosynthesis of ethylene : In plants ethylene is formed from the amino acid methionine, a constituents of all cells. In addition, certain fatty acids (Linolenic-acid) can also serve as precursors of ethylene and this shows the possibility of lipid metabolism connected to ethylene biosynthesis. Since ethylene is characterised by only in the aqueous phase of a cell, it diffuses into the atmosphere. It has been observed that synthesis of ethylene and plant response increase with an increase in temperature to an optimum level (30°C for apples). On the other hand low temperature (0 to 2°C) reduce ethylene production and plant response but do not prevent them. Ethylene synthesis (but without interfering its action) is also inhibited if oxygen concentration is low. On the other hand, at high CO_2 levels plants do not respond to ethylene.

Bioassay of ethylene : Triple response of etiolated peas has been widely used as one of the bioassays of ethylene. In this triple response the etiolated pea seedlings treated by different concentrations (.005-.64 ppm) show three different responses of growth-inhibition of elongation, increase in stem diameter and transverse geotropism. Such finding were observed when pea seeds germinated in dark for 3 days were passed over with ethylene at a rate of 30 cc h^{-1} for 47h in treatment chambers. Now gas-chromatography assist in measuring even small amounts of ethylene (as low as 1 ppb) from plant organs and tissues.

Mechanism of ethylene action : Ethylene acts by stimulating enzyme synthesis and by influencing transport of materials through membranes which in turn account for the observed responses. Recent experiments confirm that as different growth and developmental processes are susceptible to ethylene action therefore, there are as many mechanisms of ethylene action as there are modes of ethylene operation. Obviously CO_2 is a competitive inhibitor of the enzyme reactions activated by ethylene.

Commercial uses of Ethylene

Ethylene has been used for synchronised flowering and fruit ripening for centuries. Since nineteenth century, Pureto Rican and Philippine pineapple growers lit bonfire near their plantation, knowing that smoke helped to initiate and synchronise the flowering of their crops and ripening of fruits. Several chemicals, which release ethylene are used now-a-days for promotion of flowering in pineapple, all over the pine apple producing world. Etherel or Etephpri is the most commonly used chemical, which is 2-chloroethyl phosphoric acid ($Cl—CH_2—CH_2—PO_3H_2$). It rapidly breaks down in wave at neutral or alkaline pH to produce ethylene, Cl^- and PO^{-4}. This and some other uses of ethylene which are related to its physiological effects are listed.

Role of Plant Hormones or Plant Growth substances in Agriculture

This is one of the most modern and applied aspects of plant physiology. Each group of plant hormones (synthetic as well as natural) has played a very important role in the solution of several agricultural problems. Outlines of their uses in given below :

1. Rooting and propagation.
2. Control of dormancy,
3. Control of flowering,
4. Sex expression and sex modification.
5. Development of parthenocarpic fruits by use of plant growth substances.
6. Control of plant senescence.
7. Control of abscission.
8. Control of plant size and related phenomenon.
9. Weed control etc.