Review Article

A Review of Plant Growth Substances: Their Forms, Structures, Synthesis and Functions

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Abstract: Plant growth substances are compounds, either natural or synthetic that modifies or controls through physiological action, the growth and maturation of plants. If the compound is produced within the plant, it is called a plant hormone or phytohormone. In general, it is accepted that there are five major classes of plant hormones. They are Auxins (IAA), Cytokinins, Gibberellins, Ethylene and Abscisic Acid. However, there are still many plant growth substances that cannot be grouped under these classes, though they also perform similar functions, inhibiting or promoting plant growth. These substances include Brassinosteroids (Brassins), Salicylic Acid, Jasmonic Acid, Fusicoccin, Batasins, Strigolactones, Growth stimulants (e.g. Hymexazol and Pyripropanol), Defoliants (e.g. Calcium Cyanamide, Dimethipin). Researchers are still working on the biosynthetic pathways of some of these substances. Plant growth substances are very useful in agriculture in both low and high concentrations. They affect seed growth, time of flowering, the sex of flowers, senescence of leaves and fruits, leaf formation, stem growth, fruit development and ripening, plant longevity, and even plant death. Some synthetic regulators are also used as herbicides and pesticides. Therefore, attention should be paid to the production and synthesis of these substances so that they affect plants in a way that would favour yield.

Keywords: Phytohormone, Auxins, Cytokinins, Gibberellins, Ethylene, Abscisic acid, Plant Growth Regulators.

CHAPTER ONE

1. Introduction

Growth can be defined as an increase in some quantity over time. The quantity can be physical (e.g. Growth in height), abstract (e.g. a system becoming more complex, an organism becoming more mature). Growth can also refer to the quantitative change that accompanies development. Growth in plants can be defined as an irreversible increase in volume of an organism (Taiz and Zeiger, 2002).

Growth in plants is obtainable majorly at meristems where rapid mitosis provides new cells. As these cells differentiate, they provide new plant tissue. In stems, mitosis in the apical meristem of the shoot apex (also called the terminal bud) produces cells that enable the stem to grow longer and, periodically, cells that will give rise to leaves. The point on the stem where leaves develop is called a node. The region between a pair of adjacent nodes is called the Internode.

The internodes in the terminal bud are very short so that the developing leaves grow above the apical meristem that produced them and thus protect it. New meristems, the lateral buds, develop at the nodes, each just above the point where a leaf is attached. When the lateral buds develop, they produce new stem tissue, and thus branches are formed.

Under special circumstances (such as changes in photoperiod), the apical meristem is converted into a flower bud. This develops into a flower. The conversion of the apical meristem to a flower bud "uses up" the meristem so that no further growth of the stem can occur at that point. However, lateral buds behind the flower can develop into branches.

1.1 Measurement of Plant Growth

To capture enough data on the overall growth of plants, it is recommended that at least one final weight

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measure is recorded, one measure of root health, and all of the observation measurements that pertain to the type of plant being used.

1.1.1 Weighing Plants: Fresh vs. Dry Weight

Measuring Fresh Weight: While the fresh weight of plants can be technically measured without harming them, the simple act of removing a plant from its growing "medium" can cause trauma and affect the ongoing growth rate and thus the experiment. Measuring the fresh weight of plants is tricky and should probably be saved as a final measure of growth at the end of the experiment. Here is the process of measuring fresh weight; Plants are removed from the soil and washed to remove any loose soil. The plants are then blotted gently with soft paper towel to remove any free surface moisture. They are weighed immediately (plants have a high composition of water, so waiting to weigh them may lead to some drying and therefore produce inaccurate data).

Measuring dry weight: Since plants have a high composition of water and the level of water in a plant will depend on the amount of water in its environment (which is very difficult to control), using dry weight as a measure of plant growth tends to be more reliable. You can only capture this data once as a final measure at the conclusion of the experiment (Wood and Roper, 2007).

Plants are removed from the soil and washed. They are again blotted to remove any free surface moisture. The plants are then dried in an oven set to low heat (100 degrees) overnight. They are left to cool in a dry environment (a Ziploc bag will keep moisture out) - in a humid environment the plant tissue will take up water. Once the plants have cooled weigh them on a scale. Plants contain mostly water, so a scale that goes down to milligrams should be used since a dry plant will not weight very much.

1.1.2 Root Mass

Root mass is recommended as a final measurement as the plant must be removed from its growing medium in order to capture accurate data. There are quite a few different methods for measuring root mass depending on the type and structure of the roots.

Grid intersects technique: The plant is removed from the soil. When working with thin or light roots, the roots may be dyed using an acidic stain. The roots are then laid on a grid pattern this is then followed by counting the number of times the roots intersect the grid. The roots are traced on paper, with each of the tracings measured, and the root length is calculated from the tracings. The numbers of roots are then counted and the diameter measured. This is especially useful for root vegetables such as beets, carrots and potatoes that have a large root (Mulanax, 2005).

1.1.3 Root Shoot Ratio

Roots allow a plant to absorb water and nutrients from the surrounding soil, and a healthy root system is key to a healthy plant. The root: shoot ratio is one measure to help assess the overall growth of the plants. The control group of plants will provide a "normal" root: shoot ratio for each of the plant types, any changes from this normal level (either up or down) would be an indication of a change in the overall health of the plant. It is important to combine the data from the root: shoot ratio with data from observations to get an accurate measurement. For example, an increase in root: shoot ratio could be an indication of a healthier plant, provided the increase came from greater root size and NOT from a decrease in shoot weight. To measure the root: shoot ratio:

The plants are removed from soil and washed to remove any loose soil. They are blotted to remove any free surface moisture. They are then dried in an oven set to low heat (100 degrees) overnight. The plants are left to cool in a dry environment (a Ziploc bag will keep moisture out) - in a humid environment the tissue will take up water. Once the plants have cooled, they are weighed on a scale.

The root is separated from the top (cut at the soil line), then both are weighed and recorded separately for each plant. (Dry weight for roots/dry weight for top of plant = root/shoot ratio). The root/shoot ratio can be calculated for each treatment. Plants contain mostly water, so make sure you have a scale that goes down to milligrams since a dry plant will not weight very much (MBG, 2003).

1.2 Plant Growth Substances

A plant growth regulator is an organic compound, either natural or synthetic, that modifies or controls one or more specific physiological processes within a plant. If the compound is produced within the plant it is called a plant hormone. A plant regulator is defined by the Environmental Protection Agency as "any substance or mixture of substances intended, through physiological action, to accelerate or retard the rate of growth or maturation, or otherwise alter the behaviour of plants or their produce.

Additionally, plant regulators are characterized by their low rates of application; high application rates of the same compounds often are considered herbicidal (Peggy, 1999). Simply put, plant growth regulators (also known as growth regulators or plant hormones) are chemicals used to alter the growth of a plant or plant part. Hormones are substances naturally produced by plants, substances that control normal plant functions, such as root growth, fruit set and drop,

growth and other development processes. Plant growth regulators are regulated as pesticides by the Florida Department of Agriculture and Consumer Services (FDACS) and must be registered with the FDACS for lawful use in Florida like any pesticide lawfully used in Florida.

Plant hormones (also known as phytohormones) are chemicals that regulate plant growth, which, in the UK, are termed 'plant growth substances'. The term 'Phytohormone' was coined by Thimann in 1948. Plant hormones are not nutrients, but chemicals that in small amounts promote and influence the growth, development, and differentiation of cells and tissues (Opik *et al.*, 2005). Plant hormones are signal molecules produced within the plant and occur in extremely low concentrations. Hormones regulate cellular processes in targeted cells locally and when moved to other locations of the plant. Hormones also determine the formation of flowers, stems, leaves, the shedding of leaves, and the development and ripening of fruit (Salisbury and Ross, 1992).

Plants, unlike animals, lack glands that produce and secrete hormones. Instead, each cell is capable of producing hormones. Plant hormones shape the plant, affecting seed growth, time of flowering, the sex of flowers, senescence of leaves, and fruits. They affect which tissues grow upward and which grow downward, leaf formation and stem growth, fruit development and ripening, plant longevity, and even plant death. Hormones are vital to plant growth, and, lacking them,

plants would be mostly a mass of undifferentiated cells. So they are also called growth factors or growth hormones.

1.3 Forms of Plant Growth Substances

According to the American Society for Horticultural Science, plant growth regulators fall into six major classes (Fishel, 2006). These classes include Auxins, Gibberellins, Cytokinins, ethylene generators, Growth inhibitors and Growth retardants. But in general, it is accepted that there are five major classes of plant hormones, some of which are made up of many different chemicals that can vary in structure from one plant to the next. The chemicals are each grouped together into one of these classes based on their structural similarities and on their effects on plant physiology. Other plant hormones and growth regulators are not easily grouped into these classes; they exist naturally or are synthesized by humans or other organisms, including chemicals that inhibit plant growth or interrupt the physiological processes within plants. A large number of related chemical compounds are synthesized by humans. They are used to regulate the growth of cultivated plants, weeds, and *in vitro*-grown plants and plant cells; these man-made compounds are called Plant Growth Regulators or PGRs for short. Early in the study of plant hormones, "phytohormone" was the commonly used term, but its use is less widely applied now.

Table 1. Plant Growth Hormones and Regulators.

Plant Growth Hormones	Plant Growth Regulators
	Auxins
	Naphthalene acetic acid (NAA),
Auxins	2,4-Dichlorophenoxyacetic acid (2,4-D),
Indole-3-acetic acid (IAA)	2,methyl-4-chlorophenoxyacetic acid (MCPA),
Indoleacetonitrile (IAN)	2,4,5-Trichlorophenoxyacetic acid (2,4,5-T)
Indole ethanol (Iethanol)	Phenylacetic acid (PAA)
Indole pyruvic acid (IPA)	Indolepropionic acid (IPA)
	Indolebutyric acid (IBA)
	Phenoxyacetic acid (PAC)
Gibberellins	
Gibberellic acids, 1 > 100	
Cytokinins	*Cytokinins*
Zeatin, ribosyl zeatin, 6-methylaminopurine.	Kinetin, 2-Isopentenyl adenine (2, i-p), 6-Benzylaminopurine (6,BAP) or Benzyladenine
Abscisic Acid	
Ethylene	Ethylene inhibitors such as aviglycine, ethylene releasers such as etacelasil, ethephon, glyoxime.
Others Fusicoccin, Brassins, Turgorins, Salicylic Acid, Jasmonic Acid, Batasins, Plant Peptide Hormones, Polyamines, Nitric Oxide, Strigolactones and Karrikins	*Others* Gametocides (fenridazon, maleic hydrazide), Morphactins (chlorfluren, chlorflurenol, dichlorflurenol, flurenol), Growth retardants (chlormequat, daminozide, flurprimidol, mefluidide, paclobutrazol, tetcydasis, uniconazole), Growth stimulators (brassinolide, brassinolide-ethyl, forchlorfenuron, hymexazol, prosuler, pyripropanol, triacontanol), Defoliants (calcium cyanamide, dimethipin, endothal, ethephon, merphos, metoxuron, pentachlorophenol, thidiazuron, tribufos).

CHAPTER TWO

2.   Auxins and Cytokinins

2.1 Auxins

Auxins were the first class of growth regulators discovered. Indole-3-acetic acid (IAA) is the main auxin in higher plants and has profound effects on plant growth and development. Both plants and some plant pathogens can produce IAA to modulate plant growth (Zhao, 2010). The *in vitro* bioassay in which auxin-containing agar blocks stimulated the growth of oat coleoptile segments led to the identification of indole-3-acetic acid (IAA) as the main naturally occurring auxin in plants. Applications of IAA or synthetic auxins to plants cause profound changes in plant growth and development (Bonner, 1952).
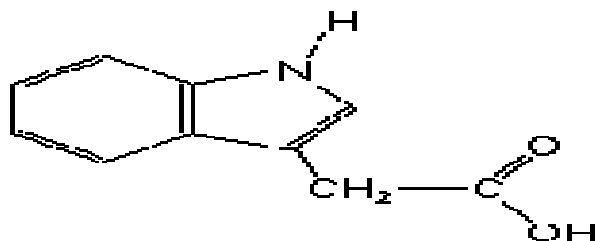
Fig. 1. Chemical Structure of Indole-3-Acetic Acid ($C_{10}H_9NO_2$).

Apart from IAA, there are other natural auxins found in plants, they are indole ethanol (Iethanol), indoleacetonitrile (IAN) and indole pyruvic acid (IPA). Synthetic auxins which are also known as man-made auxins include 2,4-dichlorophenoxyacetic acid (2,4-D), naphthalene acetic acid (NAA), indole butyric acid (IBA) etc.
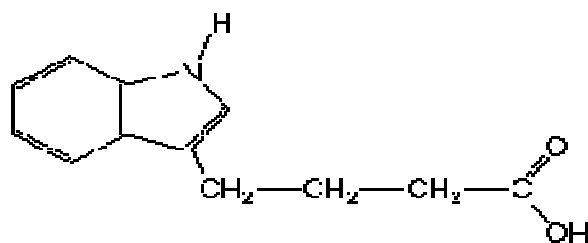

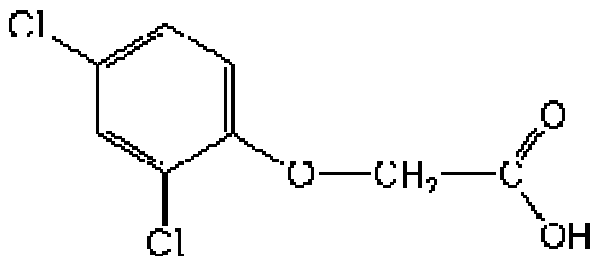Fig. 2. Chemical Structure of Indole Butyric Acid ($C_{12}H_{13}NO_2$).

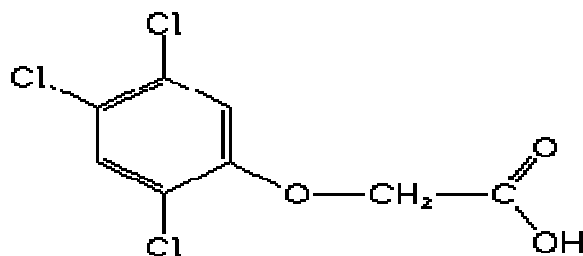Fig. 3. Chemical Structure of 2,4-dichlorophenoxyacetic acid ($C_8H_6Cl_2O_3$).

Fig. 4. Chemical Structure of 2,4,5-trichlorophenoxyacetic acid ($C_8H_5Cl_3O_3$).
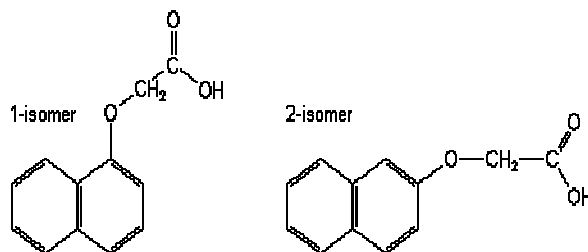

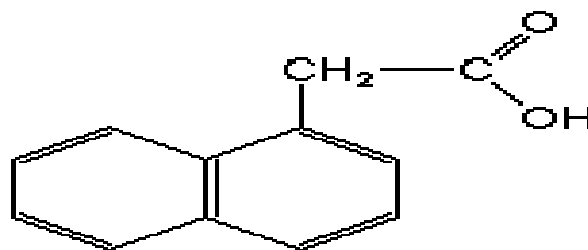Fig. 5. Chemical Structure of Naphthoxyacetic Acid ($C_{12}H_{10}O_3$).

Fig. 6. Chemical Structure of Naphthalene Acetic Acid ($C_{12}H_{10}O_2$).

2.1.1 Discovery of Auxin

The coleoptiles of grasses (like that of oat, the *Avena* - coleoptile) are popular test objects of plant physiology. A growing coleoptile that is illuminated unilaterally does grow towards the light source. This behaviour is common among plants and is known as phototropism. Charles Darwin (assisted by his son Francis Darwin) attributed in his work "The Power of Movement in Plants" (1880) a decisive function in the recognition of a light stimulus to the coleoptiles tip, and he observed that the actual bending occurs in a zone below the tip. He concluded that a transmission of impulse had to take place in the tissue. Studies of plant anatomical nature revealed that the growth towards light is caused by an elongation of the cells at the side that is shielded from the light. The phototropic reaction does not happen if the coleoptiles tip is removed, though it can be induced again by the replacement of the tip. This indicates the existence of a substance that is spread from tip to bottom (basipetal direction) and that causes the elongation.

The Danish botanist P. Boysen-Jensen interrupted the assumed substance flow by inserting a mica sheet into the shielded side, thus separating the coleoptiles tip from the tissue below (1913). The water-impermeable sheet interrupted the phototropic reaction. Consequently occurs no transport of the effector around the small tile. The phototropic reaction remained intact when the mica sheet was inserted into the illuminated side or along the coleoptiles vertical axis.

In the late twenties was the material nature of the effector finally proved by the Dutch plant physiologist F. Went. He assumed that a substance that flows from tip to bottom should also flow through a small cube of agar. In order to test his assumption did he place cut coleoptile tips with the cutting side on top of small cubes of agar. Sometime later did he remove the tips and placed the agar cubes that he believed to contain the effector onto the decapitated coleoptiles. He wrote about the carrying out of the decisive experiment:

"When I removed the tip after an hour and placed the agar cube on one side of the seedling, nothing happened at first. But in the course of the night, the stump started to curve away from the agar block. It had acquired the capacity of the stem tip to grow! At 3:00 A.M. on April 17, 1926" (Salisbury and Ross, 1978). Went called the effector auxin (or growth-regulating substance). Its chemical name is indole-3-acetic acid (IAA). The formula shows that it is a tryptophane derivative.

2.1.2 Biosynthesis of Auxin

Auxin is an essential hormone, but its biosynthetic routes in plants have not been fully defined. Auxin is an essential regulator for various plant developmental processes. Indole-3-acetic acid (IAA), the main auxin in plants, can be synthesized from tryptophan (Trp) –dependent and -independent pathways (Zhao, 2010). Several auxin biosynthesis routes have been proposed, but none of the proposed pathways in plants have been fully determined. In addition, several pathways are known to exist in the tryptophan-dependent pathway, including the tryptamine (TAM), indole-3-pyruvic acid (IPA) and indole-3-acetaldoxime (IAOx) pathways (Soeno *et al.*, 2010). Plants also make IAM, but the biosynthesis routes of IAM in plants are not defined (Sugawara *et al.,* 2009). IAM can be converted to IAA by Arabidopsis AMIDASE1 (Lehmann *et al.*, 2010). In some plant pathogenic bacteria, IAA is synthesized from Trp by the Trp monooxygenase iaaM and the hydrolase iaaH. The iaaM converts Trp to indole-3-acetamide (IAM) that is subsequently hydrolyzed to IAA by iaaH (Comai and Kosuge, 1982).

Trp can also be converted into indole-3-acetaldoxime (IAOx) by the P450 enzymes CYP79B2 and -B3 (Zhao *et al.,* 2002). The routes from IAOx to IAA are not understood, although both IAM and indole-3-acetonitrile have been suggested as intermediates (Sugawara *et al.,* 2009). IAOx is probably not the main auxin biosynthesis intermediate, because

(i)   A complete elimination of IAOx production in Arabidopsis only leads to subtle growth defects (Zhao *et al.*, 2002);

(ii)   The enzymes CYP79B2 and -B3 seemed only to exist in a small group of plants, and

(iii) There is no detectable IAOx in rice and maize (Sugawara *et al.*, 2009).

Auxin biosynthesis in plants is extremely complex. Multiple pathways likely contribute to de novo auxin production. IAA can also be released from IAA conjugates by hydrolytic cleavage of IAA-amino acids, IAA-sugar, and IAA-methyl ester (Li *et al.*, 2008). Furthermore, although plants share evolutionarily conserved core mechanisms for auxin biosynthesis, different plant species may also have unique strategies and modifications to optimize their IAA biosynthesis.
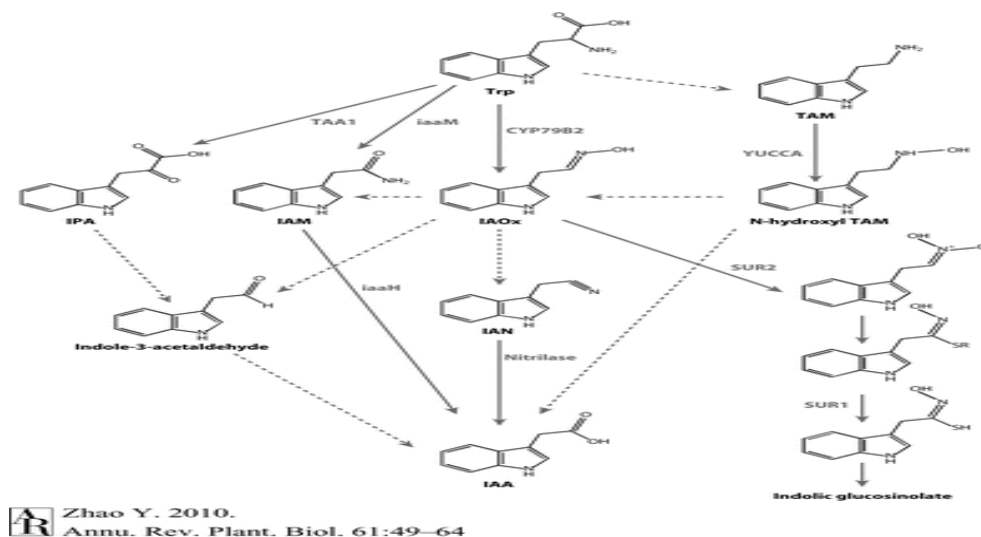

Zhao Y. 2010.
Annu. Rev. Plant. Biol. 61:49–64

Fig. 7. Biosynthesis of Auxin.

2.1.3 Auxin Transport

The distribution of auxin within plant tissues is subject to clear and recognizable rules that indicate a transport simultaneously active and polar. This means that IAA-specific carriers have to exist beside the receptor (or receptors). At least six reasons speak for this: Transport occurs always directed: it is polarized. The transport velocity is higher than expected from simple diffusion. Transport can occur against a concentration gradient. Transport is energy-consuming and is drastically reduced in the absence of oxygen. The transport system is substrate-specific. It transports certain auxin molecules like IAA or naphthylacetic acid faster than 2,4-dichlorphenoxy acetic acid, for example. The transport system can be blocked by specific inhibitors.

Experiments with plasma membrane vesicles showed that the accumulation of auxin is dependent on pH and electron potential. The transport of auxins through the membrane is directed. Auxin specifically binds tonoplasts, and it influences the release of calcium ions from the vacuole *in vitro*. Different carriers for import and export have been detected: a proton carrier (S) that causes symport and an auxin-anion-carrier (AC) that is an active antiport-carrier. Moreover, was it demonstrated that these auxin carriers are distributed asymmetrically within the membrane.

2.1.4 Auxin Functions and Physiological Effects

Auxins physiological effects in plants are highly related to its functions. These effects include Auxins are compounds that positively influence cell enlargement, bud formation and root initiation. They also promote the production of other hormones and in conjunction with cytokinins, they control the growth of stems, roots, and fruits, and convert stems into flowers (Osborne, 2005). They affect cell elongation by altering cell wall plasticity. They stimulate cambium, a subtype of meristem cells, to divide and in stems cause secondary xylem to differentiate. Auxins act to inhibit the growth of buds lower down the stems (apical dominance), and also to promote lateral and adventitious root development and growth. Leaf abscission is initiated by the growing point of a plant ceasing to produce auxins. Auxins in seeds regulate specific protein synthesis, (Walz *et al*., 2002) as they develop within the flower after pollination, causing the flower to develop a fruit to contain the developing seeds. Auxins are toxic to plants in large concentrations; they are most toxic to dicots and less so to monocots. Because of this property, synthetic auxin herbicides, including 2,4-D and 2,4,5-T have been developed and used for weed control. Auxins, especially 1-Naphthaleneacetic acid (NAA) and Indole-3-butyric acid (IBA), are also commonly applied to stimulate root growth when taking cuttings of plants.

Bending toward a light source (phototropism), downward root growth in response to gravity (geotropism), flower formation, fruit set and growth. NAA and IBA is often the active ingredient in products that stimulate root formation on plant cuttings. Auxins may be used to enhance fruit production or harvesting, but these effects are species specific. Auxin, along with cytokinin, is used in for culturing plant tissues for mass propagation. Auxins increase the plasma current, the plasticity of the cell wall, and they cause a proton efflux out of the cell. Experiments show that auxins increase the rate of transcription, control the activity of certain enzymes, and have an influence on the ion pumps within the membrane.

2.2 Cytokinins

Cytokinins are compounds with a structure resembling adenine which promotes cell division and have other similar functions to kinetin. Kinetin was the first cytokinin discovered and so named because of the compounds ability to promote cytokinesis (cell division). Though it is a natural compound, It is not made in plants and is therefore usually considered a "synthetic" cytokinin (meaning that the hormone is synthesized somewhere other than in a plant). The most common form of naturally occurring cytokinin in plants today is called zeatin which was isolated from corn (*Zea mays*).

Cytokinins have been found in almost all higher plants as well as mosses, fungi, bacteria, and also in tRNA of many prokaryotes and eukaryotes. Today there are more than 200 natural and synthetic cytokinins combined. Cytokinin concentrations are highest in meristematic regions and areas of continuous growth potential such as roots, young leaves, developing fruits and seeds (Salisbury and Ross, 1992).
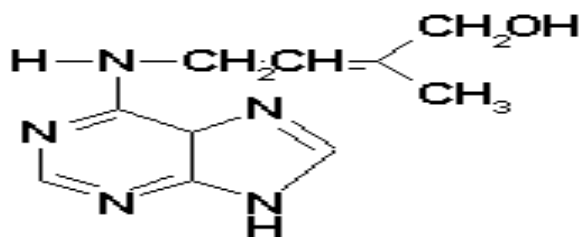

Fig. 8. Chemical Structure of Cytokinins ($C_{10}H_9N_{50}$).

2.2.1 Discovery of Cytokinins

Unlike other hormones, cytokinins are found in both plants and animals. Cytokinins or CKs are a group of chemicals that influence cell division and shoot formation. They were called kinins in the past when the first cytokinins were isolated from yeast cells. It has been tried for a long time to cultivate, plant tissue on artificial nutrient medium. The first approaches go back to the Austrian plant anatomist G. Haberlandt (1854-

1945, professor at Graz, later at Berlin). At first, posed the composition of a suitable nutrient medium large problem. The addition of coconut milk causes a drastic increase in the growth of plant embryos and tissue cultures (Overbeek, 1941). Coconut milk is an endosperm product that has under natural conditions, too, a growth-stimulating effect on the developing coconut embryo. The question which components cause the growth stimulation arose immediately.

In contrast to auxin, growth is not by cell elongation but by cell division. Aged or autoclaved DNA preparations have the same effect, while fresh DNA preparations display no effect at all (Miller and Skoog, 1955). In the end was the adenine derivative 6-furfurylaminopurine (kinetin) identified as the effective substance.

Kinetin is physiologically extraordinary active, although it could not be isolated from any plant cell. Instead was a wide range of similar compounds found. The first that was isolated from a natural source (unripe corn seed) was zeatin (Letham *et al.*, 1964).
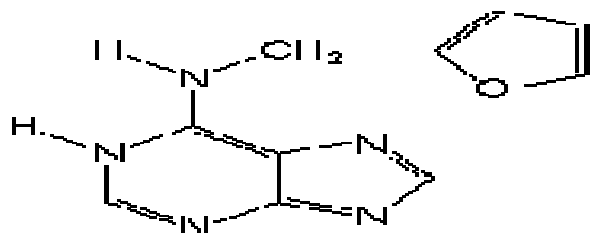

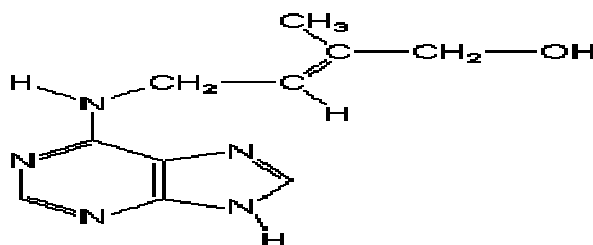**Fig. 9. Chemical Structure of Kinetin ($C_{10}H_9N_5O$).**

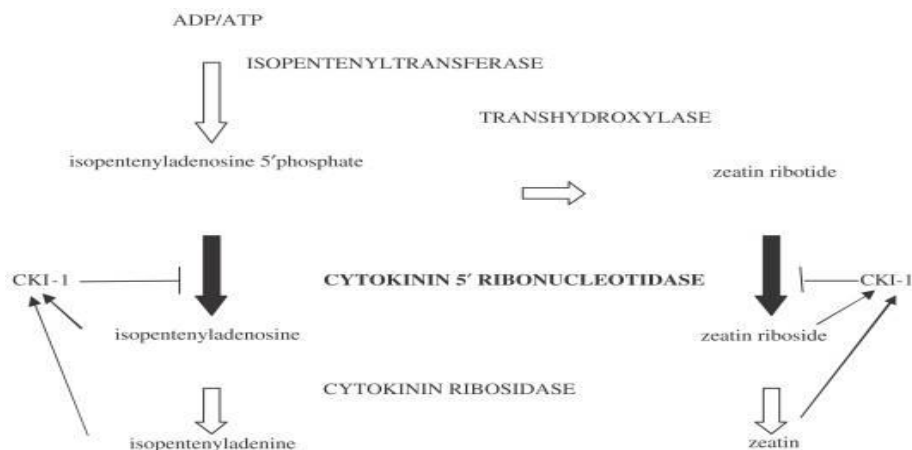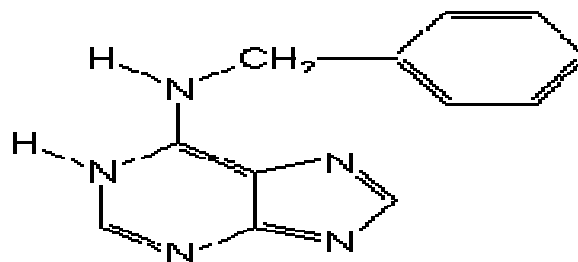**Fig. 10. Chemical Structure of Zeatin ($C_{10}H_{13}N_5O$).**

Fig. 10. Chemical Structure Of 6-Benzylaminopurine ($C_{12}H_{11}N_5$).

2.2.2 Biosynthesis and Metabolism of Cytokinins

Cytokinin is generally found in higher concentrations in meristematic regions and growing tissues. They are believed to be synthesized in the roots and translocated via the xylem to shoot. Cytokinin biosynthesis happens through the biochemical modification of adenine. The process by which they are synthesized is as follows: A product of the mevalonate pathway called isopentyl pyrophosphate is isomerized.

This isomer can then react with adenosine monophosphate with the aid of an enzyme called isopentenyl AMP synthase. The result is isopentenyl adenosine-5'-phosphate (isopentenyl AMP). This product can then be converted to isopentenyl adenosine by removal of the phosphate by a phosphatase and further converted to isopentenyl adenine by removal of the ribose group. Isopentenyl adenine can be converted to the three major forms of naturally occurring cytokinins. Other pathways or slight alterations of this one probably lead to the other forms. Degradation of cytokinins occurs largely due to the enzyme cytokinin oxidase. This enzyme removes the side chain and releases adenine. Derivatives can also be made, but the pathways are more complex and poorly understood (Salisbury and Ross, 1992).

Fig. 11. Synthesis of Cytokinins (Source: Glover *et al.*, 2008).

2.2.3 Functions and Physiological Effects of Cytokinins

They also help delay senescence or the aging of tissues, are responsible for mediating auxin transport throughout the plant and affect the internodal length and leaf growth. They have a highly synergistic effect in concert with auxins, and the ratios of these two groups of plant hormones affect most major growth periods during a plant's lifetime. Cytokinins counter the apical dominance induced by auxins; they in conjunction with ethylene promote abscission of leaves, flower parts, and fruits (Sipes and Einset, 1983). Tissue cultures like that of tobacco or maple (*Acer pseudoplatanus*) develop only after the addition of cytokinin. Besides, the rate of DNA replication, increase cytokinins, too, the general rate of RNA and protein synthesis. They reduce senescence and stimulate the dark-germination of light-dependent seeds. In addition were several more selective effects observed. Cytokinins induce isocitrate lyase and protease activities in cut pumpkin cotyledons; they induce thiamine synthesis in growing callus cultures of tobacco, thereby removing the thiamine need of the calli. Cytokinins stimulate auxin synthesis in tissue cultures of tobacco; they also increase the carboxy dismutase and the NADP-glyceraldehydes phosphate dehydrogenase activities in etiolated rice seedlings. Cytokinin stimulates the development of buds as well as the germination of seeds, and the accumulation of nitrate reductases in several embryos.

CHAPTER THREE

3.   Gibberellin, Ethylene and Abscisic Acid

3.1 Gibberellins

Gibberellins, or GAs, include a large range of chemicals that are produced naturally within plants and by fungi. They were first discovered when Japanese researchers, including Eiichi Kurosawa, noticed a chemical produced by a fungus called *Gibberella fujikuroi* that produced abnormal growth in rice plants (Grennan, 2006).

Unlike the classification of auxins which are classified on the basis of function, gibberellins are classified on the basis of structure as well as function. All gibberellins are derived from the ent-gibberellane skeleton. The structure of this skeleton derivative along with the structure of a few of the active gibberellins is shown above. The gibberellins are named GA1....GAn in order of discovery. Gibberellic acid, which was the first gibberellin to be structurally characterised, is GA3. There are currently 136 GAs identified from plants, fungi and bacteria. GAs are widespread and so far ubiquitous in flowering (angiosperms) and non-flowering (gymnosperms) plants as well as ferns.
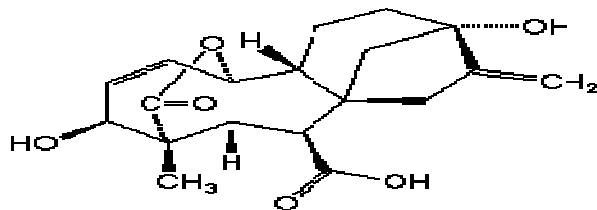

Fig. 12. Chemical Structure of Gibberellic Acid ($C_{19}H_{22}O_6$).

3.1.1 Gibberellin Biosynthesis and Metabolism

Gibberellins are diterpenes synthesized from acetyl CoA via the mevalonic acid pathway. They all have either 19 or 20 carbon units grouped into either four or five ring systems. The fifth ring is a lactone ring as shown in the structures above attached to ring A. Gibberellins are believed to be synthesized in young tissues of the shoot and also the developing seed. It is uncertain whether young root tissues also produce gibberellins. There is also some evidence that leaves may be the source of some biosynthesis (Sponsel, 1995).

Certain commercial chemicals which are used to stunt growth do so in part because they block the synthesis of gibberellins. Some of these chemicals are Phosphon D, Amo-1618, Cycocel (CCC), ancymidol, and paclobutrazol. During active growth, the plant will metabolize most gibberellins by hydroxylation to inactive conjugates quickly with the exception of GA3. GA3 is degraded much slower, which helps to explain why the symptoms initially associated with the

hormone in the disease bakanae are present. Inactive conjugates might be stored or translocated via the phloem and xylem before their release (activation) at the proper time and in the proper tissue (Sponsel, 1995).

3.1.2 Functions and Physiological Effects of Gibberellins

Gibberellins are important in seed germination, affecting enzyme production that mobilizes food production used for growth of new cells. This is done by modulating chromosomal transcription. In grain (rice, wheat, corn, etc.) seeds, a layer of cells called the aleurone layer wraps around the endosperm tissue. Absorption of water by the seed causes production of GA. The GA is transported to the aleurone layer, which responds by producing enzymes that break down stored food reserves within the endosperm, which are utilized by the growing seedling. Gas produces bolting of rosette-forming plants, increasing internodal length. They promote flowering, cellular division, and in seed growth after germination. Gibberellins also reverse the inhibition of shoot growth and dormancy induced by Abscisic Acid (Tsai *et al.,* 1997). Gibberellins also stimulate stem elongation by stimulating cell division and elongation. They stimulate bolting/flowering in response to long days, break seed dormancy in some plants which require stratification or light to induce germination. Stimulates enzyme production (a-amylase) in germinating cereal grains for mobilization of seed reserves. Gibberellins induce maleness in dioecious flowers (sex expression) and they cause parthenocarpic (seedless) fruit development. They also delay senescence in leaves and citrus fruits.

3.2 Ethylene

Ethylene is unique in that it is found only in the gaseous form and it is often found in high concentrations within cells at the end of a plant's life. Ethylene is a gas that forms through the Yang Cycle from the breakdown of methionine, which is in all cells. Ethylene has a very limited solubility in water and does not accumulate within the cell, but diffuses out of the cell and escapes out of the plant. Its effectiveness as a plant hormone is dependent on its rate of production versus its rate of escaping into the atmosphere. Ethylene is produced at a faster rate in rapidly growing and dividing cells, especially in darkness. New growth and newly germinated seedlings produce more ethylene than can escape the plant, which leads to elevated amounts of ethylene (Wang *et al*., 2007).


Fig. 13. Chemical Structure of Ethylene (C_2H_4).

3.2.1 Biosynthesis of Ethylene

In higher plants, ethylene is produced from L-methionine. Methionine is activated by ATP to form S-adenosylmethionine through the catalytic activity of S-adenosylmethionine synthetase. Starting from S-adenosylmethionine two specific steps result in the formation of ethylene. The first step produces the non-protein amino acid, 1-aminocyclopropane-1-carboxylic acid (ACC). It is catalyzed by ACC synthase with pyridoxal phosphate acting as a co-factor. Formation of ACC is the rate-limiting step in ethylene biosynthesis. ACC synthase is encoded by a medium-size multigene family. Various signals, which influence ethylene synthesis, result in increased expression of single members of the ACC synthase gene family. Production of ethylene from ACC is catalyzed by ACC oxidase. This reaction is oxygen-dependent. At anaerobic conditions, ethylene formation is completely suppressed. Fe^{2+} is a co-factor and ascorbate cosubstrate; CO_2 was shown to activate ACC oxidase. ACC oxidases are encoded by small gene families in plants.

The concentration of ethylene in a plant tissue is dependent on the rate of biosynthesis and on the diffusion of the gas. Ethylene is neither actively transported nor degraded. Induction of ethylene synthesis by signals such as auxin or wounding usually occurs through activation of ACC synthase through increased gene expression. ACC oxidase activity, on the other hand, is constitutively present in most vegetative plant tissues. In some cases, further induction of ACC oxidase by ethylene is observed (Sauter, 2012). In maturing fruits, ethylene synthesis is autocatalytically enhanced, i.e., ethylene induces its own biosynthesis. The self-enhancing synthesis and diffusion of the gaseous hormone throughout the fruit accelerate ripening and contribute to a synchronized
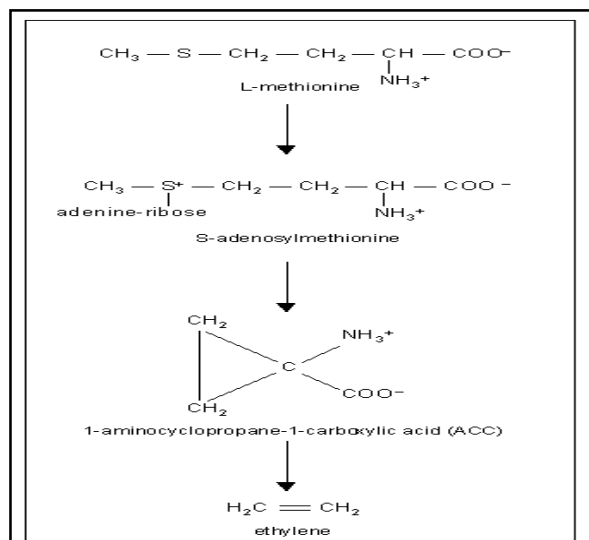
ripening process.

Fig. 14. Biosynthesis of Cytokinins (Source: McKeon *et al.*, 1995).

3.2.2 Ethylene Distribution and Transport

Probably each plant tissue produces or takes up ethylene at some point during development and responds to it in an appropriate fashion. Uptake is usually not a problem because ethylene can diffuse freely through membranes. The distribution of the gas within the plant occurs through intercellular spaces and when dissolved in the symplast from cell to cell. Long-distance distribution is achieved by releasing ACC into the vascular tissue where it is moved to the site of action. There, is converted to ethylene. Tomatoes, for instance, produce ACC in water-logged roots. ACC is moved *via* the vascular bundles to the leaves where it is converted to ethylene, which then induces epinastic lowering of the petioles (Sauter, 2012).

3.2.3 Functions and Physiological Effects of Ethylene

Leaf expansion: Ethylene affects cell growth and cell shape; when a growing shoot hits an obstacle while underground, ethylene production greatly increases, preventing cell elongation and causing the stem to swell. The resulting thicker stem can exert more pressure against the object impeding its path to the surface. If the shoot does not reach the surface and the ethylene stimulus becomes prolonged, it affects the stem's natural geotropic response, which is to grow upright, allowing it to grow around an object. Studies seem to indicate that ethylene affects the stem diameter and height: When stems of trees are subjected to wind, causing lateral stress, greater ethylene production occurs, resulting in thicker, more sturdy tree trunks and branches. Ethylene affects fruit-ripening: Normally, when the seeds are mature, ethylene production increases and builds-up within the fruit, resulting in a climacteric event just before seed dispersal (Wang *et al*., 2007).

3.3 Abscisic Acid

Abscisic acid also called ABA, was discovered and researched under two different names before its chemical properties were fully known, it was called *dormin* and *abscisin II*. Once it was determined that the two latter compounds are the same, it was named abscisic acid. The name "abscisic acid" was given because it was found in high concentrations in newly abscissed or freshly fallen leaves.

This class of PGR is composed of one chemical compound normally produced in the leaves of plants, originating from chloroplasts, especially when plants are under stress. In general, it acts as an inhibitory chemical compound that affects bud growth, and seed and bud dormancy. It mediates changes within the apical meristem, causing bud dormancy and the

alteration of the last set of leaves into protective bud covers. Since it was founded in freshly abscissed leaves, it was thought to play a role in the processes of natural leaf drop, but further research has disproven this. Plant species from temperate parts of the world, it plays a role in leaf and seed dormancy by inhibiting growth, but, as it is dissipated from seeds or buds, growth begins. In other plants, as ABA levels decrease, growth then commences as gibberellin levels increase. Without ABA, buds and seeds would start to grow during warm periods in winter and be killed when it froze again. Since ABA dissipates slowly from the tissues and its effects take time to be offset by other plant hormones, there is a delay in physiological pathways that provide some protection from premature growth. It accumulates within seeds during fruit maturation, preventing seed germination within the fruit, or seed germination before winter. Abscisic acid's effects are degraded within plant tissues during cold temperatures or by its removal by water washing in out of the tissues, releasing the seeds and buds from dormancy (Feurtado *et al*., 2004).

In plants under water stress, ABA plays a role in closing the stomata. Soon after plants are water-stressed and the roots are deficient in water, a signal moves up to the leaves, causing the formation of ABA precursors there, which then move to the roots. The roots then release ABA, which is translocated to the foliage through the vascular system (Ren *et al*., 2007). It then modulates the potassium and sodium uptake within the guard cells, which then lose turgidity, closing the stomata (Yan *et al*., 2007). ABA exists in all parts of the plant and its concentration within any tissue seems to mediate its effects and function as a hormone; its degradation, or more properly catabolism, within the plant affects metabolic reactions and cellular growth and production of other hormones (Kermode, 2005). Plants start life as a seed with high ABA levels. Just before the seed germinates, ABA levels decrease; during germination and early growth of the seedling, ABA levels decrease even more. As plants begin to produce shoots with fully functional leaves, ABA levels begin to increase, slowing down cellular growth in more "mature" areas of the plant. Stress from water or predation affects ABA production and catabolism rates, mediating another cascade of effects that trigger specific responses from targeted cells. Scientists are still piecing together the complex interactions and effects of this and other phytohormones.
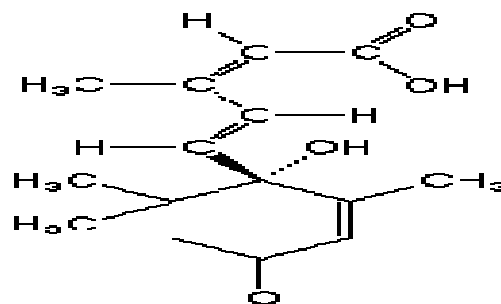

Fig. 15. Chemical Structure of Abscisic acid ($C_{15}H_{20}O_4$).

CHAPTER FOUR

4. Other Plant Growth Substances

4.1 Lunularic Acid

In liverworts, lunularic acid is present in gemmae. Lunularic acid prevents germination of these gemmae until they fall from the mother plant's thallus and the acid is leached out (Milborrow, 1984). Furthermore, the growth of the entire thallus seems to be partly controlled lunularic acid in response to day length. During short days the concentration of the inhibitor is low and thalli grow rapidly, during long days the reverse is true. Lunularic acid is present in many species of lower plants, but not in algae (Gorham, 1977).
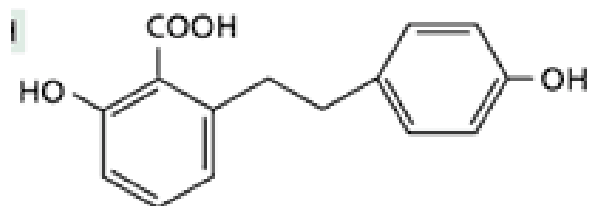

Fig. 16. Structure of lunularic acid.

4.2 Batasins

These are compounds found in yam plants (*Dioscorea batatas*) that seem to cause dormancy in bulbils (vegetative reproductive structures) that arise from the swelling of the aerial lateral buds. Batasins are concentrated in the skin of the bulbils and are absent from the core. A long duration of exposure to cold temperature (stratification or pre-chilling) that breaks dormancy causes batasins to disappear, whereas their amounts increase during the initial development of dormant bulbils (Hasegawa and Hashimoto, 1975). However, it is not known whether batasins are accumulated in or transported into the bud cells whose failure to grow actually causes dormancy.

4.3 Jasmonic Acid

Jasmonic acid and its methyl ester (methyl jasmonate) occur in several plant species and in the oil of jasmine (Parthier, 1990). Jasmonates have been found in over 150 families and 260 species including fungi, mosses and ferns, so they are perhaps ubiquitous in plants. JA is a lipid derived plant hormone, which is structurally similar to prostaglandins in animals (Solomon *et al.*, 2011).

Jasmonic acid (JA) is derived from the fatty acid linolenic acid. It is a member of the jasmonate class of plant hormones. It is biosynthesized from linolenic acid by the octadecanoid pathway. JA has been shown to inhibit growth of certain plant parts and to strongly promote leaf senescence (Salisbury and Ross, 1992). The major function of JA and its various metabolites is regulating plant responses to abiotic and biotic stresses as well as plant growth and development (Delker *et al.*, 2006). Regulated plant growth and development processes include growth inhibition, senescence, tendril coiling, flower development and leaf abscission.
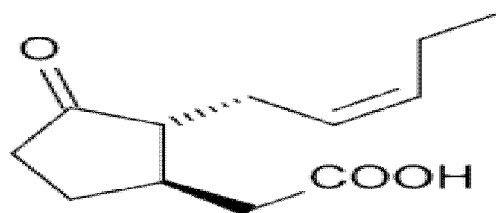

Fig. 17. Chemical Structure of Jasmonic Acid ($C_{12}H_{18}O_3$).

JA is also responsible for tuber formation in potatoes, yams, and onions. It has an important role in response to wounding of plants and systemic acquired resistance. When plants are attacked by insects, they respond by releasing JA, which activates the expression of protease inhibitors, among many other anti-herbivore defense compounds. These protease inhibitors prevent the proteolytic activity of the insects' digestive proteases, thereby stopping them from acquiring the needed nitrogen in the protein for their own growth (Zavala *et al.,* 2004).

For example, research on Madagascar periwinkle (*Catharanthus roseus*), which makes some anti-cancer alkaloids, identified a transcription factor that responds to JA by activating the expression of several genes encoding alkaloid biosynthetic genes (Van der Fits and Memelink, 2000). This chemical may have a role in pest control, according to an October 2008 BBC News report. JA affects several plant processes, such as pollen development, root growth, fruit ripening and senescence and it is also produced in response to the presence of insect pests and disease-causing organisms. JA triggers the production of enzymes that confer an increased resistance against herbivorous (plant eating) insects. For example, caterpillar infested tomato plants release volatile jasmonic acid into the air that attracts natural caterpillar enemies such as parasitic wasps. The wasps lay their eggs on the caterpillars' bodies, when the eggs hatch, the wasp larvae consume the host insects. In one study, plants treated with jasmonic acid increased parasitism on caterpillar pests two folds over control fields that were not treated with it.

Biologists have made progress in identifying key aspects of the jasmonic acid signalling pathway. Interestingly the jasmonic acid pathway includes ubiquitin as in the auxin signalling pathway. The

ubiquitin tags in the JA signalling pathway cause degradation of transcription receptors, thereby altering gene expression (Solomon *et al.,* 2011).

4.4 Brassinosteroids

Brassinosteroids (also called brassins) are plant steroid hormones, although steroid hormones in plants were not well characterised until the mid 1990s. They have distinctive growth promoting activity in some plants, especially in stems. These substances were first isolated from bee-collected pollen grains of rape (*Brassica napus*), mustard (Salisbury and Ross, 1992). The structure of one brassin known as brassinolide is shown below.
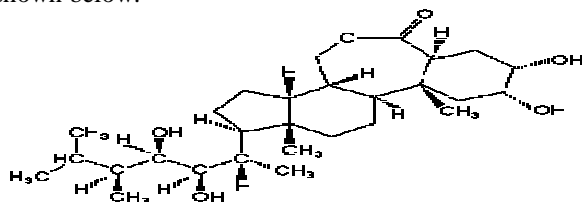

Fig. 18. Chemical Structure of Brassinolide ($C_{28}H_{48}O_6$).

The brassinosteroids (BRs) are involved in several aspects of growth and development. Arabidopsis mutants that cannot synthesize BRs are dwarf plants with small curly leaves and reduced fertility. This effect can be reversed by researchers by applying BR exogenously to the plant. Studies of these mutants suggest that BRs are involved in multiple developmental processes such as cell division, vascular development and seed germination.

Many steps of the BR signalling pathway have been characterised. BRs bind to a receptor kinase in the plant plasma membrane (unlike steroid hormones in animals which enter the cell and bind to receptors in the cytoplasm or nucleus). When BR binds to its receptor, the receptor phosphorylates molecules in the cytosol, which in turn triggers a signalling cascade that ultimately alters gene expression in the cell. Part of the signal transduction process involves attaching ubiquitin to nuclear proteins targeted for degradation in a proteasome. As a result, the expression of BR target genes is adjusted (Solomon *et al*., 2011).
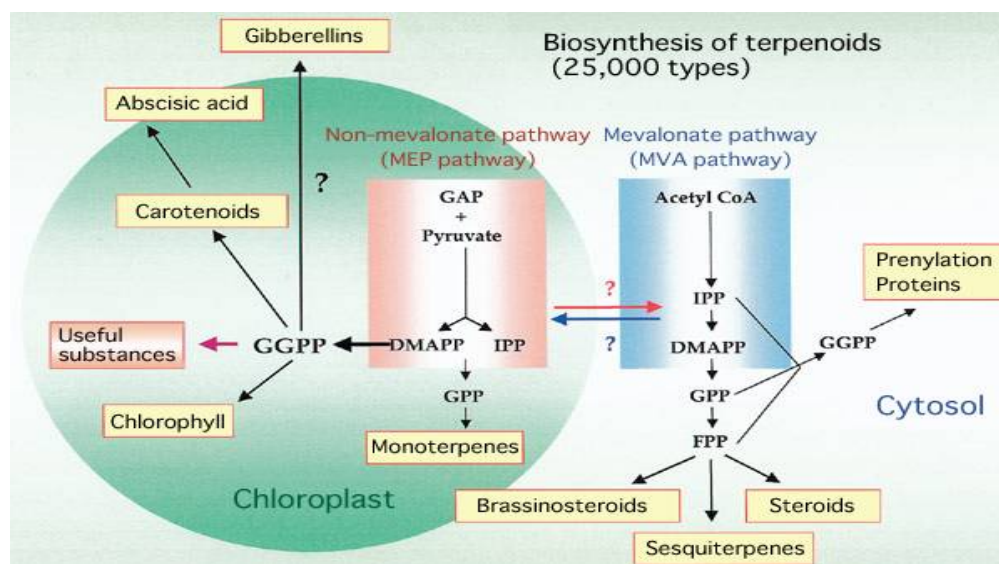

Fig. 19. Biosynthesis of Brassinosteroids, Abscisic Acid and Gibberellins (Source: Gianpiero, 2009).

4.5 Salicylic Acid

Salicylic acid, a phenolic extract from willow bark, was long used as an analgesic. It is now prepared commercially and is the active ingredient of aspirin.

Salicylic acid is known to activate defense genes for resistance against insect pests and pathogen invaders, known as the hypersensitive response.

When a plant is under attack, the concentration of SA increases and it spreads systemically throughout the plant. Plants also use methyl salicylate, which is volatile to signal nearby plants. Tobacco plants infected with Tobacco Mosaic Virus release methyl salicylate into the air. When nearby healthy plants receive the airborne chemical signal, they begin synthesizing anti-viral proteins that enhance their resistance to the virus (Solomon *et al*., 2011).
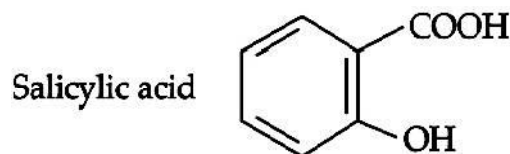

Fig. 20. Chemical structure of salicylic acid ($C_7H_6O_3$).

4.5.1 Pathways of SA biosynthesis in plants

Isotope feeding experiments suggest that plants synthesize SA from cinnamate produced by phenylalanine ammonia lyase (PAL). Genetic studies have indicated that the bulk of SA is produced from isochorismate. Given the importance of both PAL and ICS in SA accumulation demonstrated from experiments using genetic mutants, gene silencing and chemical inhibition, it is possible that the PAL and isochorismate synthase (ICS) pathways are integrated through a metabolic or regulatory grid in SA biosynthesis. The recently identified PBS3 and EPS1 are important for pathogen-induced SA production and may encode enzymes catalyzing related, and possibly sequential, reactions in the synthesis of an important precursor or regulatory molecule for SA biosynthesis (Zhixiang *et al*., 2009).
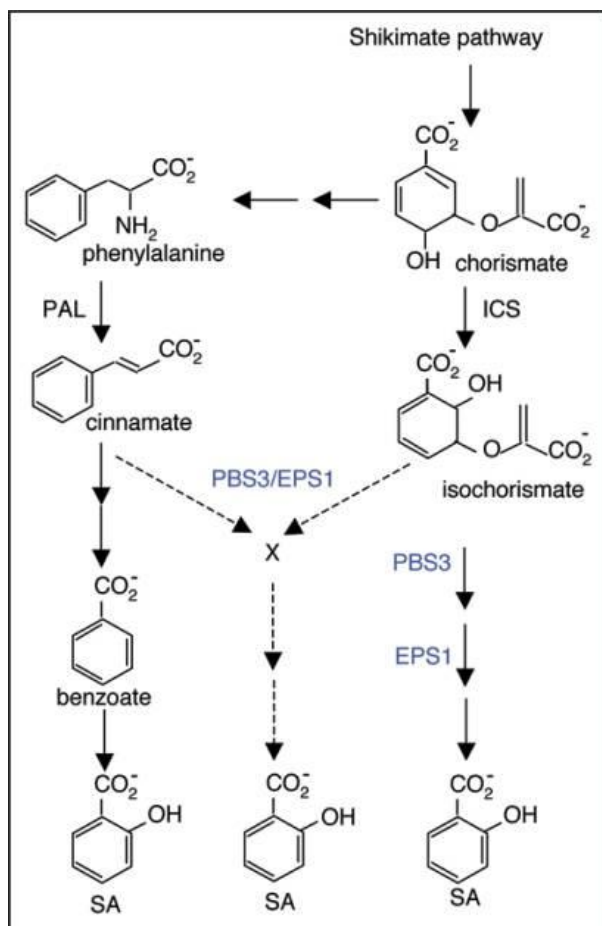

Fig. 21. Biosynthetic Pathway of Salicylic Acid.

4.6 Fusicoccin

Fusicoccin is a diterpene glucoside that was identified by plant pathologists in the 1960s as the principal toxin responsible for disease symptoms caused by the fungus *Fusicoccum amygdali* on peach, almond, and prune trees (Marre, 1979). It is a fungal phytotoxin that stimulates both rapid proton extrusion and transient growth in the stem and coleoptile sections (Taiz and Zeiger, 2002). Research with both cucumber cotyledons and maize coleoptiles verified that fusicoccin is a potent growth promoter and can acidify cell walls enough to promote their growth. It has the remarkable abilities to activate a plasma membrane ATPase that transports hydrogen ion from the cytosol into the wall to lower the wall pH, to enhance wall loosening and to promote cell growth.

Even though fusicoccin can increase growth of coleoptiles and cotyledons as it promotes hydrogen ion efflux, auxins cannot promote hydrogen ion efflux enough to promote maize-coleoptile growth, nor can cytokinins promote hydrogen ion efflux enough to enhance cotyledon growth. This implies that, in effect, auxins and other hormones must cause wall loosening and cell expansion in some (maybe most) species by some mechanism (Salisbury and Ross, 1992).

4.7 Tugorins

These sets of compounds were discovered by Schildknecht (1983, 1984) using leaves of the plant *Mimosa* and *Acacia karroo*. He isolated and identified compounds that activated pulvini in these leaves which are not sensitive to touch but exhibits nyctinasty.

A *Mimosa* leaf is placed in a solution with a suspected active substance which is then transported in the transpiration stream to the pulvini, where their membranes respond and cause the leaf pinnules to fold up if the substance is active. Two Periodic Leaf Movement Factors (PLMFs) from *Acacia* proved to be the β-glucosides of Gallic acid, with the glucoside bonding at its para-hydroxyl group. Several other compounds with similar structures were also identified from other plants. The most active were the β-D-glucoside-6-sulphate (called PLMF1) and the β-D-glucoside-3,6-sulphate (PLMF 2) of Gallic acid. Extracts from *Mimosa* contained PLMF 1 and PLMF 7, another Npound called PLMF 3 which is derived from protocatechuic acid were isolated from *Oxalis stricta* (this species react to touch or shaking as much as *Mimosa* does). Glucose-6-sulphate was found to be part of the molecule. Schildknecht then suggested that these compounds form a new class of plant hormones, which he called Tugorins because they act on turgor of pulvinus cells, and they are active at low concentrations like other phytohormones (Salisbury and Ross, 1992).

4.8 Strigolactones

Strigolactones are plant hormones that stimulate the branching and growth of symbiotic arbuscular mycorrhizal fungi, increasing the probability of contact and establishment of a symbiotic association between the plant and fungus (López-Ráez *et al.*, 2009). Strigolactones inhibit plant shoot branching (Gomez-

Roldan *et al.,* 2008). They also trigger germination of parasitic plant seeds (for example *Striga* from which they gained their name) (Akiyama and Hayashi, 2006). Strigolactones are carotenoid-derived and contain a labile ether bond that is easily hydrolyzed in the rhizosphere meaning that there is a large concentration gradient between areas near the root and those further away. Recently, strigolactone biosynthesis were found to be DWARF27 dependent (Lin *et al.,* 2009).
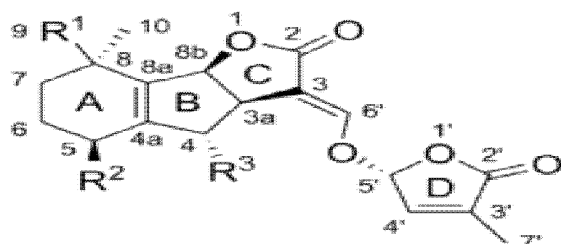

Fig. 22: Chemical Structure of Strigolactones (C₁₇H₁₄O₅).

4.9 Karrikins

Karrikins are a chemically defined family of plant growth regulators discovered in smoke from burning plant material. Karrikins are potent in breaking dormancy of seeds of many species adapted to environments that regularly experience fire and smoke. The recent discovery that karrikins trigger seed germination and control seedling growth in taxa that would rarely experience fire indicates that their significance could extend far beyond fire ecology. This is exemplified by new studies showing that the seeds of

Arabidopsis thaliana respond sensitively and specifically to karrikins in smoke. These exciting discoveries might be explained if karrikins are produced in the environment by processes other than fire, such as by chemical or microbial degradation of vegetation in response to disturbance of the soil or removal of the plant canopy. Another hypothesis is that plants contain endogenous karrikins that function naturally in the control of seed germination and that species from fire-prone habitats have evolved to respond also to exogenous karrikins. A variant on this hypothesis is that karrikins mimic endogenous plant hormones such as terpenoids that control seed germination. The evidence for these hypotheses is discussed, but whatever the explanation karrikins are now firmly established as an important family of naturally occurring plant growth regulators.
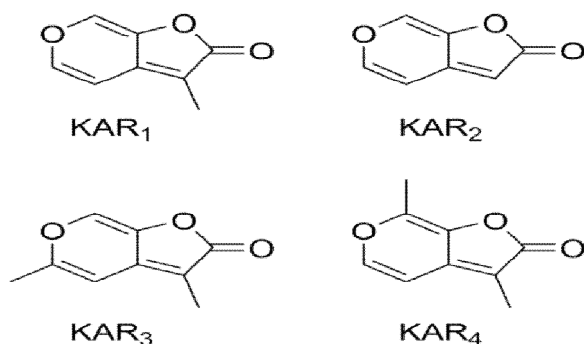

Fig. 23. Chemical Structure of Karrikins.

CHAPTER FIVE

4. Conclusion

Growth is essential in the life of any living organism and it is usually controlled or regulated by one or more factors, either internal (within the organism) or external (its environment). In plants, these factors are achieved through the action of some chemical agents called hormones; they are triggering agents or substances because they initiate a biochemical process which ultimately leads to growth. These processes include formation of leaves, stems, flowers, roots and other characteristics of the plant. Some of these substances are also synthesized by man so they are generally referred to as plant growth substances.

Plant growth substances or regulators (PGRs) play vital roles in a plant's life from dormancy to senescence; therefore they are important in agriculture, horticulture and other areas where plant growth is essential. Some of the unclear biosynthetic pathways of these substances should also be clarified in order to maximise their potential.

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