Unit II

Pharmacognosy & Phytochemistry (BP405T)

By

Dr. Kamran Javed Naquvi,

Associate Professor,

Faculty of Pharmaceutical Sciences,

Rama University, Kanpur

UNIT-II (Part 3)

Polyploidy, mutation and hybridization with reference to medicinal plants

Plant hormones and their applications.

Polyploidy

Plants whose cells contain two sets of chromosomes, derived at fertilization from the union of one set from the pollen and one set from the egg cells, are described as diploids and denoted by "2n". The term polyploidy is applied to plants with more than two sets of chromosomes in the cells; when four sets are present the plants are described as tetraploids and denoted by "4n".

Types

Organ-specific patterns of endopolyploidy (from 2x to 64x) in the giant ant Dinoponera australis. Polyploid types are labeled according to the number of chromosome sets in the nucleus. The letter x is used to represent the number of chromosomes in a single set:

- Haploid (one set; 1x)
- Diploid (two sets; 2x)
- Triploid (three sets; 3x), for example sterile saffron crocus, or seedless watermelons, also common in the phylum Tardigrada.
- Tetraploid (four sets; 4x), for example Salmonidae fish, the cotton *Gossypium hirsutum*
- Pentaploid (five sets; 5x), for example Kenai Birch (*Betula papyrifera* var. *kenaica*)
- Hexaploid (six sets; 6x), for example wheat, kiwifruit
- Heptaploid or septaploid (seven sets; 7x)
- Octaploid or octoploid, (eight sets; 8x), for example Acipenser (genus of sturgeon fish), dahlias
- Decaploid (ten sets; 10x), for example certain strawberries
- Dodecaploid (twelve sets; 12x), for example the plants *Celosia argentea* and *Spartina anglica* or the amphibian *Xenopus ruwenzoriensis*.

Tetraploidy is induced by treatment with colchicine, which inhibits spindle formation during cell division, so that the divided chromosomes are unable to separate and pass to the daughter cells. The two sets of chromosomes remain in one cell and this develops to give tetraploids plant.

Treatment with colchicine may be applied in various ways, but all depend on the effects produced in the meristem. The seeds may be soaked in a dilute solution of colchicine, or the seedlings, the soil around the seedling or the young shoot treated with colchicine solution. Fertile seed and robust, healthy tetraploid plants were obtained, the tetraploid condition being indicated by the increased size of the pollen grains and stomata; chromosome counts in root-tip preparations confirm the tetraploid condition.

The average increase in alkaloids content compared with diploid plants of *Datura stromonium* and *Datura tatula* was 68%, with a maximum increase of 211.6%. Similar results were obtained with *Atropa belladonna* and *Hyoscyamus niger*, the average increase in belladonna being 93%. Increased Alkaloidal content of tetraploids plants has been confirmed for *Datura stromonium* and *Datura tatula*. The diploid of *Acorus calamus* is 2.1% of volatile oil content but they are converted into tetraploid, they produce 6.8% of volatile oil contents.

Mutation

Define:

Sudden heritable change in the structure of a gene on chromosome or change the chromosome number. A mutation is a change in a DNA sequence. Mutations can result from DNA copying mistakes made during cell division, exposure to ionizing radiation, exposure to chemicals called mutagens, or infection by viruses. Germ line mutations occur in the eggs and sperm and can be passed on to offspring, while somatic mutations occur in body cells and are not passed on.

Type of mutations:

1. Spontaneous and induced mutations.
2. Recessive and dominant mutations.
3. Somatic and germinal mutations.
4. Forward, back and suppressor mutation.
5. Chromosomal, genomic and point mutations.

Mutations can be artificially produced by certain agents called mutagens or mutagenic agent. They are two types:

a. Physical mutagens:

(i) Ionizing radiations:

X-rays, gamma radiation and cosmic rays.

(ii) Non-ionizing radiation:

U.V. radiation,

b. Chemical mutagens:

(iii) Alkylating and hydroxylating agents:

Nitrogen and sulpher mustard; methyl and ethylsulphonate, ethylethane sulphonates.

(iv) Nitrous acid:

(v) Acridines:

Acridines and proflavins. Ionizing radiation cause breaks in the chromosome. These cells then show abnormal cell divisions. If these include gametes, they may be abnormal and even die prematurely. Non-ionizing radiation like Ultra Violet rays are easily absorbed by purine and pyrimidines. The changed bases are known as photoproducts. U.V. rays cause two changes in pyrimidine to produce pyrimidine hydrate and pyrimidine dimmers. Thymine dimer is a major mutagenic effect of U.V. rays that disturbs DNA double helix and thus DNA replication.

Example:

Penicillin, as an antibiotic was first obtained from Penicillium. However, the yield was very poor and the preparation was commercially expensive. Since then mutants with higher yield of penicillin have been selected and produced. *Penicillium chrysogenum* used in the production of penicillin yielded about 100 units of penicillin per ml of culture medium.

By single-spore isolation, strains were obtained which yielded up to 250 units per ml of medium, X-ray treatment of this strain gave mutants which produce 500 units per ml and ultraviolet mutants of latter gave strain which produced about 1000 unit per ml. Similarly improvements have been obtained with other antibiotic- producing organism. Mutant strains of Capsicum annum with increasing yields (20-60%) of capsaicin have been isolated from M_3 and M_4 generations originating from seed treated with sodium azide and ethyl methane sulphonate.

Hybridization

It is mating or crossing of two genetically dissimilar plants having desired genes or genotypes and bringing them together into one individual called hybrid. The process through which hybrids are produced is called hybridization.

Hybridization particularly between homozygous strains, which have been inbred for a number of generations, introduces a degree of heterozygosis with resultant hybrid vigour often manifest in the dimensions and other characteristic of the plants. A hybrid is an organism which results from crossing of two species or varieties differing at least in one set of characters.

The following steps are involved in hybridization of plant:

1. Choice of parents:

The two parents to be selected, at least one should be as well adopted and proven variety in the area. The other variety should have the characters that are absent in the first chosen variety.

2. Emasculation:

Removal of stamens or anthers or killing the pollen grains of a flower without affecting the female reproductive organs is known as emasculation. Emasculation is essential in bisexual flowers.

3. Bagging:

Immediately after emasculation, the flowers or inflorescences are enclosed in bags of suitable sizes to prevent random cross-pollination.

4. Pollination:

In pollination, mature, fertile and viable pollens are placed on a receptive stigma. The procedure consists of collecting pollens from freshly dehisced anthers and dusting them on the stigmas of emasculated flowers.

5. Raising F_1 plants:

Pollination is naturally followed by fertilization. It results in the formation of seeds. Mature seeds of F_1 generation are harvested dried and stored these seeds are grown to produce F_1 hybrid. Hybrids of cinchona yield more amount of quinine. A hybrid developed by crossing Cinchona succirubra with Cinchona ledgering yields a bark, which contains 11.3% of alkaloids. The parent species produced 3.4% and 5.1% of alkaloids, respectively.

Pyrethrum hybrids have been used for Pyrethrum production; these hybrids are produced either by crossing two clones assumed to be self- sterile or planting a number of desirable clones together and bulking the seed. The hybridization of plant to increase the Pyrethrin contents

Green house effect

Normal conditions sun rays reach the earth and heat is radiated back into space. However, when carbon dioxide concentration increases in the atmosphere, it forms a thick cover and prevents the heat from being re-radiated. Consequently, the atmosphere gets heated and the temperature increases.

This is called green house effect. In recent past, amount of carbon dioxide has increased from 290 ppm to 330 ppm due to cutting of forests and excessive burning of fossil fuels. The rate at which the amount of carbon dioxide in the atmosphere is increasing, it is expected to cause rise in global temperature.

The global warming by two or three degrees would cause polar ice caps to melt, floods in coastal areas, change in hydrologic cycle and islands would get submerged. The following gases produce green house effect like carbon dioxide, sulphur dioxide, oxide of nitrogen, chlorofluorocarbons, etc.

Plant hormones and their applications

Plant Growth Regulators are defined as small, simple chemicals produced naturally by plants to regulate their growth and development. Hormones are produced naturally by plants, while plant growth regulators are applied to plants by humans. Plant hormones always play an important role as a signaling molecule and induce various plant stress responses in the perception, transduction, and induction phases of the stress response. Gibberellins, being one of the most important and primary plant hormones, have physiological functions such as stimulating organ growth through enhancement of cell elongation and cell division; they also act as a developmental switch between seed dormancy and germination, juvenile and adult growth phases, and vegetative and reproductive development. Plant growth regulators may be synthetic compounds, such as IBA and Cycocel, that mimic naturally occurring plant hormones, or they may be natural hormones that were extracted from plant tissue, such as IAA. Applied concentrations of these substances usually are measured in parts per million (ppm) and in some cases parts per billion (ppb). These growth-regulating substances most often are applied as a spray to foliage or as a liquid drench to the soil around a plant's base. Generally, their effects are short-lived, and they may need to be reapplied in order to achieve the desired effect.

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

Characteristics

There are five groups of plant-growth-regulating compounds: auxin, gibberellin (GA), cytokinin, ethylene, and abscisic acid (ABA). For the most part, each group contains both naturally occurring hormones and synthetic substances. They are also referred to as plant growth substances, phytohormones or plant hormones.

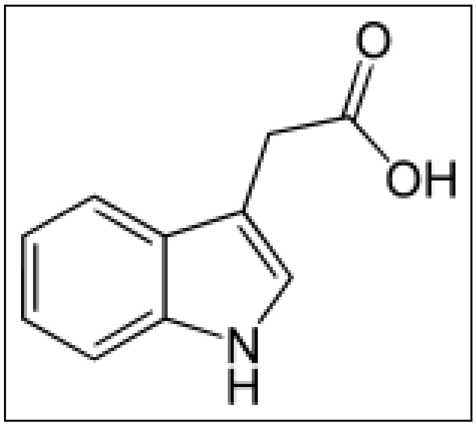
Based on their action, they are broadly classified as follows:

Plant Growth Promoters – They promote cell division, cell enlargement, flowering, fruiting and seed formation. Examples are auxins, gibberellins and cytokinins.

Plant Growth Inhibitors – These chemicals inhibit growth and promote dormancy and abscission in plants. An example is an abscisic acid.

Note: Ethylene can be a promoter or an inhibitor, but is largely a Plant Growth Inhibitor.

Auxins



Auxins are hormones produced in immature parts of plants that stimulate growth. Auxins were the first plant hormones discovered and have been studied extensively. Auxins are most commonly found in seed embryos, apical meristems and young leaves. The seed embryo has yet to develop, and the cells have not yet become differentiated - that is, the young cells don't know what they'll be when they grow up yet. Similarly, the apical meristem is the location of primary plant growth and contains new cells that have not yet decided what to become.

Auxins stimulate cell differentiation, which means this hormone helps decide if a cell will become ground tissue or vascular tissue or protective tissue. Auxins also help stimulate stem elongation, which is what primary stem growth is all about. Primary stem growth will occur when a high enough amount of auxins target a given area. Auxins also help regulate fruit development. Without auxins, fruits are often too small. Some produce farmers will spray artificial auxins on plants - such as apples or pears - in order to increase the size of the fruits.

Discovery

Auxins were the first growth hormone to be discovered. They were discovered due to the observations of Charles Darwin and his son, Francis Darwin. The Darwins observed that the coleoptile (protective sheath) in canary grass grows and bends towards the source of light. This phenomenon is 'phototropism'. In addition, their experiments showed that the coleoptile tip was the site responsible for the bending. Finally, this led to the isolation of the first auxin by F. W. Went from the coleoptile tip of oat seedlings.

Types

First isolated from human urine, auxin is a term applied to natural and synthetic compounds that have growth regulating properties. Plants produce natural auxins such as Indole-3-acetic acid (IAA) and Indole butyric acid (IBA). Natural auxins are found in growing stems and roots from where they migrate to their site of action. Naphthalene acetic acid (NAA) and 2, 4-dichlorophenoxyacetic (2, 4-D) are examples of synthetic auxins.

Effects

Bending toward a light source (phototropism).

- Downward root growth in response to gravity (geotropism).
- Promotion of apical dominance (the tendency of an apical bud to produce hormones that suppress the growth of the buds below it on the stem).
- Promote flowering in plants.
- Help to initiate rooting in stem cuttings.
- Prevent dropping of fruits and leaves too early.
- Promote natural detachment (abscission) of older leaves and fruits.
- Control xylem differentiation and help in cell division.
- Fruit set and growth.
- Formation of adventitious roots.

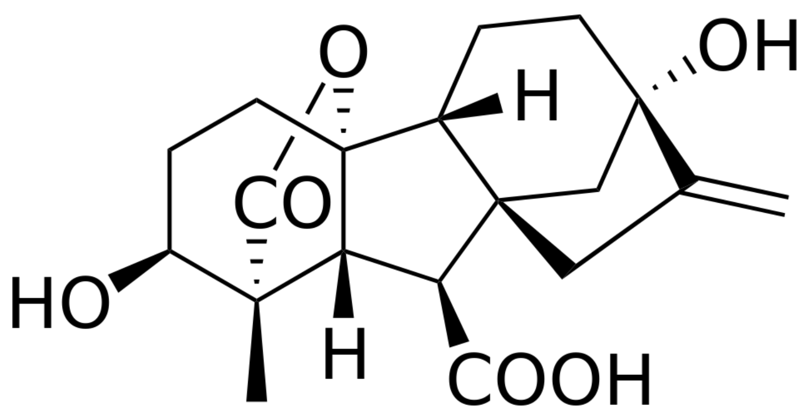
Auxin is the active ingredient in most rooting compounds in which cuttings are dipped during vegetative propagation.

Applications

- Used for plant propagation.
- To induce parthenocarpy i.e. the production of fruit without prior fertilization.
- 2, 4-D is widely used as a herbicide to kill dicotyledonous weeds.
- Used by gardeners to keep lawns weed-free.

Note: The growing apical bud in higher plants inhibits the growth of the lateral buds. This phenomenon is ‘Apical Dominance‘. Removal of the apical bud allows the lateral buds to grow. This technique is commonly used in tea plantations and hedge-making.

GIBBERELLINS



Gibberellins stimulate cell division and elongation, break seed dormancy, and speed germination. The seeds of some species are difficult to germinate; you can soak them in a GA solution to get them started. Gibberellins (GAs) include a large range of chemicals that are produced naturally within plants and by fungi. The synthesis of GA is strongly upregulated in seeds at germination and its presence is required for germination to occur. In seedlings and adults, GAs strongly promote cell elongation. GAs also promote the transition between vegetative and reproductive growth and are also required for pollen function during fertilization.

Discovery

It is the component responsible for the 'bakane' disease of rice seedlings. The disease is caused by the fungal pathogen Gibberella fujikuroi. E. Kurosawa treated uninfected rice seedlings with sterile filtrates of the fungus and reported the appearance of disease symptoms. Finally, the active substance causing the disease was identified as gibberellic acid.

Types

There exist more than 100 gibberellins obtained from a variety of organisms from fungi to higher plants. They are all acidic and are denoted as follows – GA1, GA2, GA3 etc. GA3 (Gibberellic acid) is the most noteworthy since it was the first to be discovered and is the most studied.

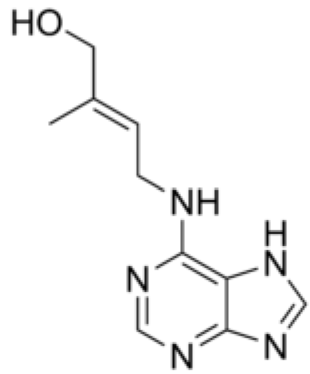
Effects

- Increase the axis length in plants such as grape stalks.
- Delay senescence (i.e. ageing) in fruits. As a result, their market period is extended.
- Help fruits like apples to elongate and improve their shape.

Applications

- The brewing industry uses GA3 to speed the malting process.
- Spraying gibberellins increase sugarcane yield by lengthening the stem.
- Used to hasten the maturity period in young conifers and promote early seed production.
- Help to promote bolting (i.e. sudden growth of a plant just before flowering) in cabbages and beet.

Cytokinins



The cytokinin, zeatin

Cytokinins are hormones that are produced in the roots, stimulate growth and have anti-aging effects. Because they're produced in roots, cytokinins must travel up through the plant's xylem in order to reach target areas, such as the stems and leaves. Cytokinins have several responsibilities, including working with auxins to stimulate growth and cell differentiation in both stems and roots. Cytokinins also specifically promote growth and development of chloroplasts, the cell organelle responsible for photosynthesis.

A unique role of cytokinins is to produce anti-aging effects on some plant parts. When you think of anti-aging products, you may think of expensive face creams that advertise giving a younger, brighter look. Cytokinins actually provide a younger, healthier look in plants. Florists commonly use cytokinins to make cut flowers look fresh for longer. By adding this hormone to cut flowers, florists are able to slow down the aging process, providing us with prettier flowers for longer.

Discovery

F. Skoog and his co-workers observed a mass of cells called ‘callus’ in tobacco plants. These cells proliferated only when the nutrient medium contained auxins along with yeast extract or extracts of vascular tissue. Skoog and Miller later identified the active substance responsible for proliferation and called it kinetin.

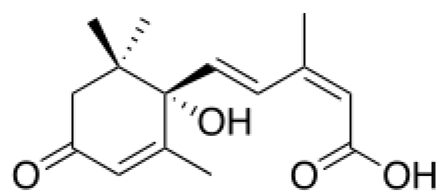
Types

Cytokinins were discovered as kinetin. Kinetin does not occur naturally but scientists later discovered several natural (example – zeatin) and synthetic cytokinins. Natural cytokinins exist in root apices and developing shoot buds – areas where rapid cell division takes place.

Effects

- Help in the formation of new leaves and chloroplast.
- Promote lateral shoot growth and adventitious shoot formation.
- Help overcome apical dominance.
- Promote nutrient mobilisation which in turn helps delay leaf senescence.

Abscisic Acid



Abscisic acid (also called ABA) is one of the most important plant growth inhibitors. It was discovered and researched under two different names before its chemical properties were fully known, it was called dormin and abscicin II. Once it was determined that the two 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.

Discovery

Three independent researchers reported the purification and characterization of three different inhibitors – Inhibitor B, Abscission II and Dormin. Later, it was found that all three inhibitors were chemically identical and were, therefore, together were given the name abscisic acid. Abscisic acid mostly acts as an antagonist to Gibberellic acid.

Effects

- Regulate abscission and dormancy.
- Inhibit plant growth, metabolism and seed germination.
- Stimulates closure of stomata in the epidermis.
- It increases the tolerance of plants to different kinds of stress and is, therefore, called ‘stress hormone’.
- Important for seed development and maturation.
- It induces dormancy in seeds and helps them withstand desiccation and other unfavourable growth factors.

Ethylene

Ethylene is a gas that forms through the breakdown of methionine, which is in all cells. Ethylene has 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, inhibiting leaf expansion. As the new shoot is exposed to light, reactions mediated by phytochrome, the plant's cells produce a signal for ethylene production to decrease, allowing leaf expansion. Ethylene affects cell growth and cell shape; when a growing shoot or root hits an obstacle while underground, ethylene production greatly increases, preventing cell elongation and causing the stem to swell. The resulting thicker stem is stronger and less likely to buckle under pressure as it presses 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 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.

Discovery

A group of cousins showed that a gaseous substance released from ripe oranges hastens the ripening of unripe oranges. Consequently, they found that the substance was ethylene – a simple gaseous Plant Growth Regulator. Ripening fruits and tissues undergoing senescence produce ethylene in large amounts.

Effects

- Affects horizontal growth of seedlings and swelling of the axis in dicot seedlings.
- Promotes abscission and senescence, especially of leaves and flowers.
- Enhances respiration rate during ripening of fruits. This phenomenon is ‘respiratory climactic’.
- Increases root growth and root hair formation, therefore helping plants to increase their absorption surface area.

Application

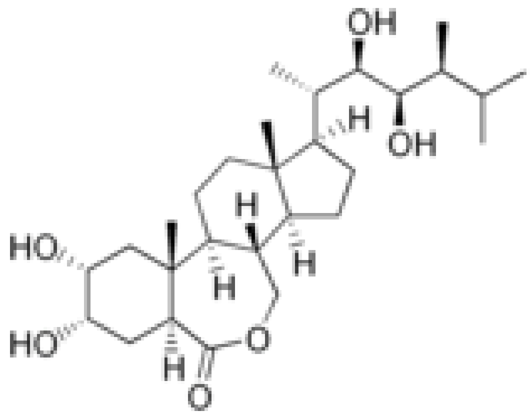
Ethylene regulates many physiological processes and is, therefore, widely used in agriculture. The most commonly used source of ethylene is Ethephon. Plants can easily absorb and transport an aqueous solution of ethephon and release ethylene slowly.

- Used to break seed and bud dormancy and initiate germination in peanut seeds.
- To promote sprouting of potato tubers.
- Used to boost rapid petiole elongation in deep water rice plants.
- To initiate flowering and synchronising fruit-set in pineapples.
- To induce flowering in mango.

Ethephon hastens fruit ripening in apples and tomatoes and increases yield by promoting female flowering in cucumbers. It also accelerates abscission in cherry, walnut and cotton.

In summary, one or the other plant growth regulator influences every phase of growth or development in plants. These roles could be individualistic or synergistic; promoting or inhibiting. Additionally, more than one regulator can act on any given life event in a plant. Along with genes and extrinsic factors, plant growth regulators play critical roles in plant growth and development. Factors like temperature and light affect plant growth events (vernalisation) via plant growth regulators.

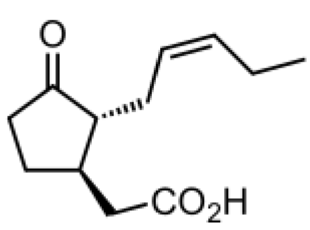
Brassinosteroids



Brassinolide, a major brassinosteroid

Brassinosteroids are a class of polyhydroxysteroids, the only example of steroid based hormones in plants. Brassinosteroids control cell elongation and division, gravitropism, resistance to stress, and xylem differentiation. They inhibit root growth and leaf abscission. Brassinolide was the first identified brassinosteroid and was isolated from extracts of rapeseed (*Brassica napus*) pollen in 1979.

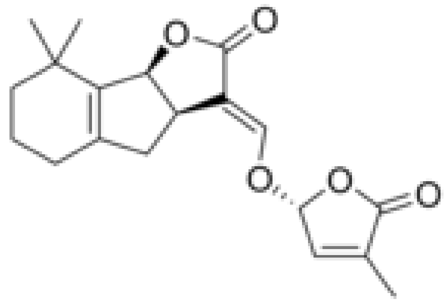
Jasmonates



Jasmonic acid

Jasmonates (JAs) are lipid-based hormones that were originally isolated from jasmine oil. JAs are especially important in the plant response to attack from herbivores and necrotrophic pathogens. The most active JA in plants is jasmonic acid. Jasmonic acid can be further metabolized into methyl-JA, which is a volatile organic compound. This unusual property means that methyl-JA can act as an airborne signal to communicate herbivore attack to other distant leaves within one plant and even as a signal to neighboring plants. In addition to their role in defense, JAs are also believed to play roles in seed germination, the storage of protein in seeds and root growth.

Strigolactones



5-deoxystrigol, a strigolactone

Strigolactones (SLs) were originally discovered through studies into the germination of the parasitic weed *Striga lutea*. It was found that the germination of Striga species was stimulated by the presence of a compound exuded by the roots of its host plant. It was later shown that SLs that are exuded into the soil promote the growth of symbiotic arbuscular mycorrhizal (AM) fungi. More recently, another role of SLs was identified in the inhibition of shoot branching. This discovery of the role of SLs in shoot branching led to a dramatic increase in the interest in these hormones, and it has since been shown that SLs play important roles in leaf senescence, phosphate starvation response, salt tolerance and light signaling.