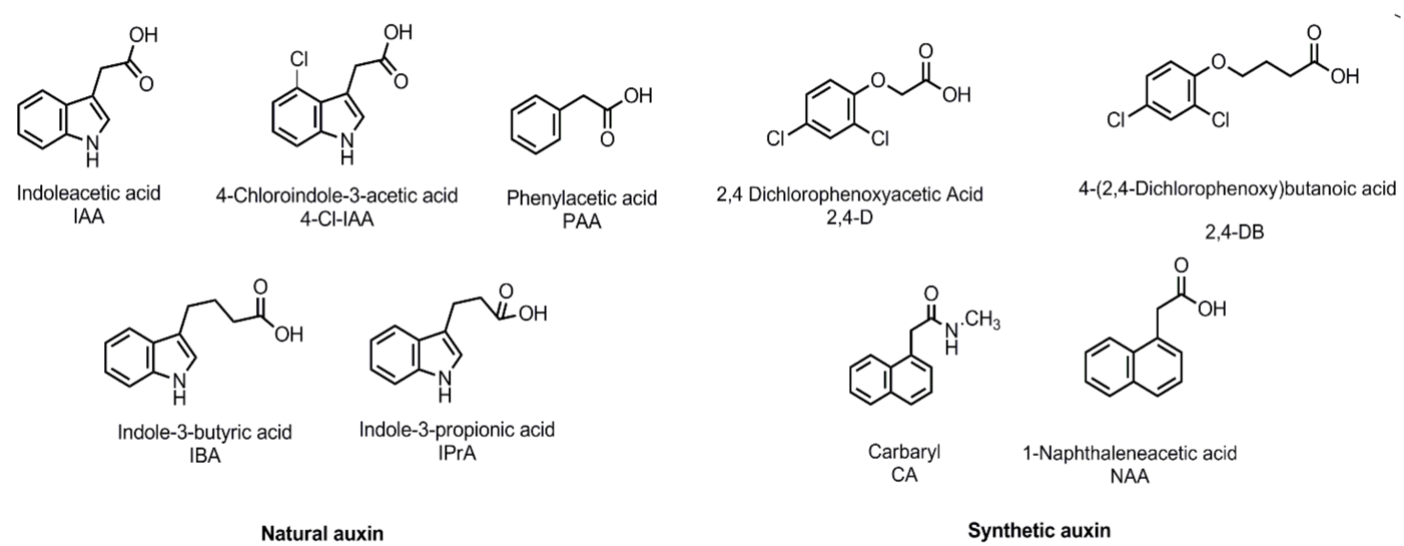
1 AUXIN (AUXs)

In 1881, Charles Darwin and his son published the book "The Power of Movement in Plants", where they studied the phenomenon of phototropism in plants (Darwin, 1880). In 1926, Frits Went continued with the study growth stimulus, evidencing the presence of a growth-promoting chemical compound that accumulated on the side opposite the illuminated zone, being the first AUX identified (Thimann, 1940). Indole-3-acetic acid (IAA) was considered as the most important AUX due to its plant-growth regulating potential and physiological relevance, being initially isolated from human urine (Kögl et al., 1934) and interestingly not from plants; many other compounds have been used as auxins including indole-3- butyric acid (IBA), indole-3-propionic acid (IPrA), and naphthalene acetic acid (NAA), among others.

The term auxin is derived from the **Greek** word '**auxein'** which means to **grow**. They are a class of plant hormones which has a cardinal role in coordination of many growth and behavioral processes in the plant's life cycle essential for development of plant. Auxin is the **first** plant hormone to be **identified**. They have the ability to induce **cell elongation** in stems and resemble indole acetic acid (the first auxin to be isolated) in physiological activity. The most widespread auxin is ***indoleacetic acid***, or simply **IAA**.

Types of Auxin

Auxins are divided into two categories Natural auxins and Synthetic auxins



Natural auxins: The four naturally occurring (endogenous) auxins are Indole-3-acetic acid, 4-chloroindole-3-acetic acid, phenyl acetic acid and indole-3-butyric acid; only these four are **synthesized by plants**.

Synthetic auxins: Synthetic auxin **analogs** include 1-naphthaleneacetic acid, 2,4-dichlorophenoxyacetic acid (2,4-D) and many others. Some synthetic auxins, such as **2,4-D** and 2,4,5-trichlorophenoxyacetic acid (**2,4,5-T**), are used also as **herbicides**.

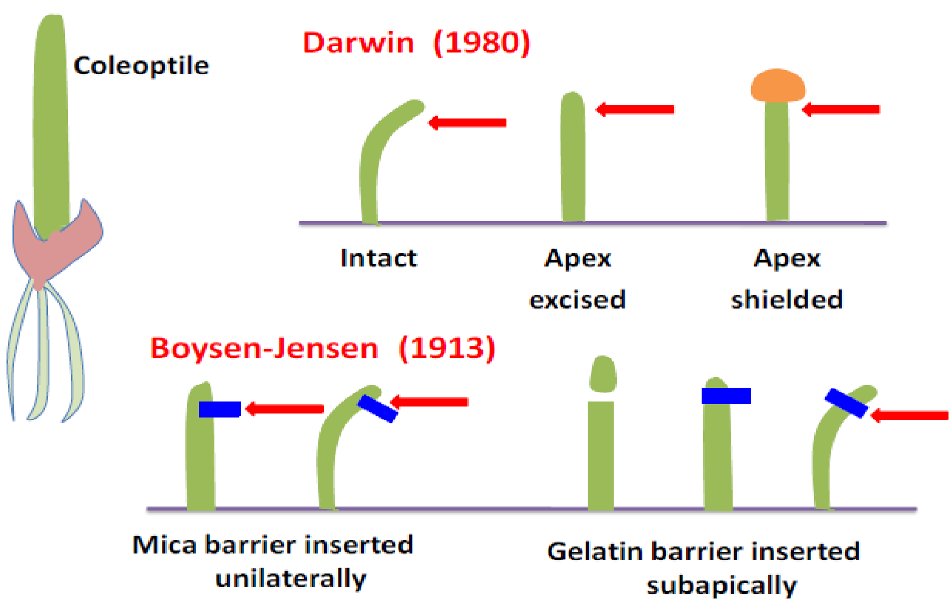
Broad-leaf plants (**dicots**), such as dandelions, are much more **susceptible** to auxins than **narrow-leaf plants** (monocots) such as grasses and cereal crops, so these synthetic auxins are useful as synthetic herbicides.

1.1 Discovery of auxin:

Darwin (1881) was the first person who **discovered** the existence of auxin in plants, the **first phytohormone** known. He noted that the **first leaf** (**coleoptile**) of **canary** grass (*Phalaris canariensis*) was very **sensitive** and **responsive to light** and he demonstrated the **bending** of the grass coleoptiles towards **unilateral source of light**. This bending occurred only when the coleoptile was also **illuminated**. When the tip of the coleoptiles was **covered** with a **black cap**, it resulted in **loss of sensitivity** of the plant towards the light. Darwin concluded that ***some influence that causes curvature is transmitted from the coleoptile tip to the rest of the shoot***.

Boysen – Jensen (1913) also made **similar** observations on **oat** (***Avena***) coleoptiles.

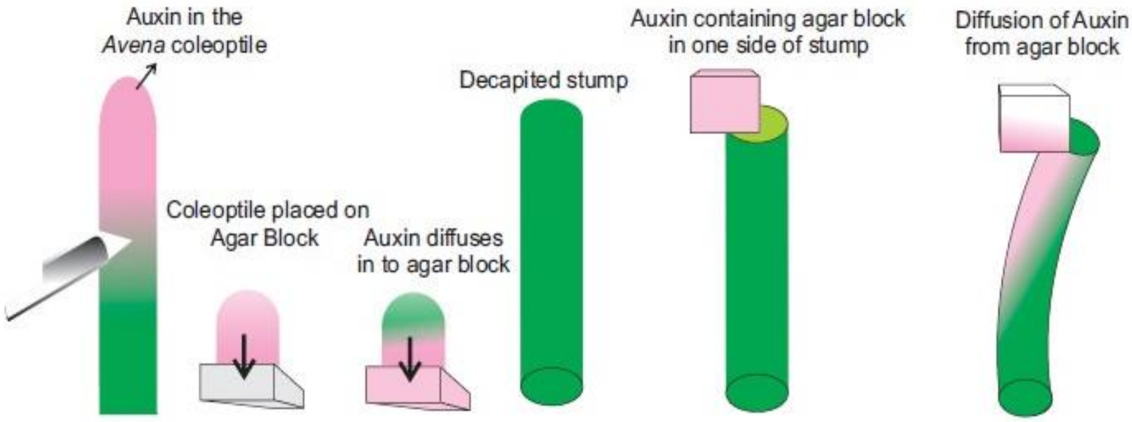
Paal (1918) demonstrated that when the **decapitated coleoptiles** tip was **replaced** on the **cut end eccentrically**, more growth resulted on the side which causes bending even when this is done in complete **darkness**.



Went (1928) isolated auxin from the **Avena coleoptile** tips by a **method** called ***Avena coleoptile or curvature test*** and concluded that ***no growth can occur without auxin***. Auxins are widely distributed throughout the plant however, abundant in the **growing tips** such as **coleoptile tip**, **buds**, **root tips** and **leaves**.

Avena coleoptile or curvature test

When the **Avena seedlings** have attained a **height** of **15 to 30 mm**, about **1mm** of the coleoptile tip is **removed**. This apical part is the **source of natural auxin**. The tip is now placed on **agar blocks for few hours**. During this period, the auxin diffuses out of these tips into the agar. The auxin containing **agar block** is now **placed on one side** of the decapitated stump of Avena coleoptile. The auxin from the agar blocks **diffuses** down through coleoptile along the side to which the auxin agar block is placed. An **agar block without auxin** is placed **on another decapitated coleoptile**. Within an hour, the coleoptiles ***with auxin agar block bends on the opposite side*** where the agar block is placed.



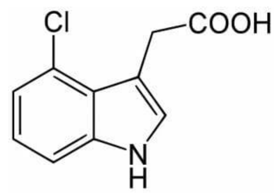
Auxin stimulates cell elongation on the shady side of the stem through a process called the acid growth hypothesis: Auxin causes cells to activate proton pumps, which then pump protons out of the cells and into the space between the plasma membrane and the cell wall. The movement of protons into the extracellular space does two things:

1. The lower pH activates expansin, which breaks the links between the cellulose fibers in the cell walls, making them more flexible.
2. The high concentration of protons causes sugars to move into the cell, which then creates an osmotic gradient where water moves into cell causing the cell to expand.

To sum up, the phototropic response works like this: the phototropins phot1 and phot2 are present in the plant apical meristem. When activated by blue light, phot1 and phot2 cause accumulation of auxin on the shaded side of the plant. Auxin promotes cell elongation due to weakening of the cell wall combined with influx of water (which literally stretches the cells). Because the cell expansion occurs only on the shaded side of the stem, the plant bends away from the shade and toward the light.

1.2 Auxin Structure

Native auxin molecules are normally derived from the **amino acid** **tryptophan**. This amino acid has a **six-sided** carbon ring, attached to a **5-sided ring** containing carbon. This 5-sided ring has a **group attached**. The only difference between most auxin molecules and tryptophan is **what is attached to this ring**. The common auxin IAA can be seen below.



1.3 Transport in Plants

Auxin is in **polar** transport. It includes **basipetal** and **acropetal** transport. **Basipetal** means transport through **phloem** from shoot to root and **acropetal** means transport through **xylem** from root to shoot.

Polar **transport** of auxin is **inhibited** by 2, 3, 5 Triiodobenzoic acid (**TIBA**) and Naphthyl thalamic acid (**NPA**). The substances are called as **antiauxins**.

1.4 Distribution of auxin in plants

In plants, auxin (**IAA**) is **synthesized** in growing tips or **meristematic regions** from where; it is transported to other plant parts. Hence, the **highest concentration** of IAA is found in growing shoot **tips**, **young leaves** and developing **auxiliary shoots**.

In **monocot** **seedling**, the highest concentration of auxin is found in **coleoptile tip** which decreases progressively towards its base.

In **dicot** **seedlings**, the highest concentration is found in growing regions of **shoot**, **young leaves** and developing **auxiliary shoots.**

Within the plants, auxin may present in two forms. i.e., ***free*** *auxins* and ***bound*** *auxins*.

Free auxins are those which are easily **extracted** by various **organic solvents** such as **diethyl ether**.

Bound auxins on the other hand, need more **drastic methods** such as **hydrolysis**, **autolysis**, **enzymolysis** etc. for extraction of auxin. Bound auxins occur in plants as **complexes** with **carbohydrates** such as glucose, arabionse or **sugar alcohols** or **proteins** or **amino acids** such as aspartate, glutamate or with inositol.

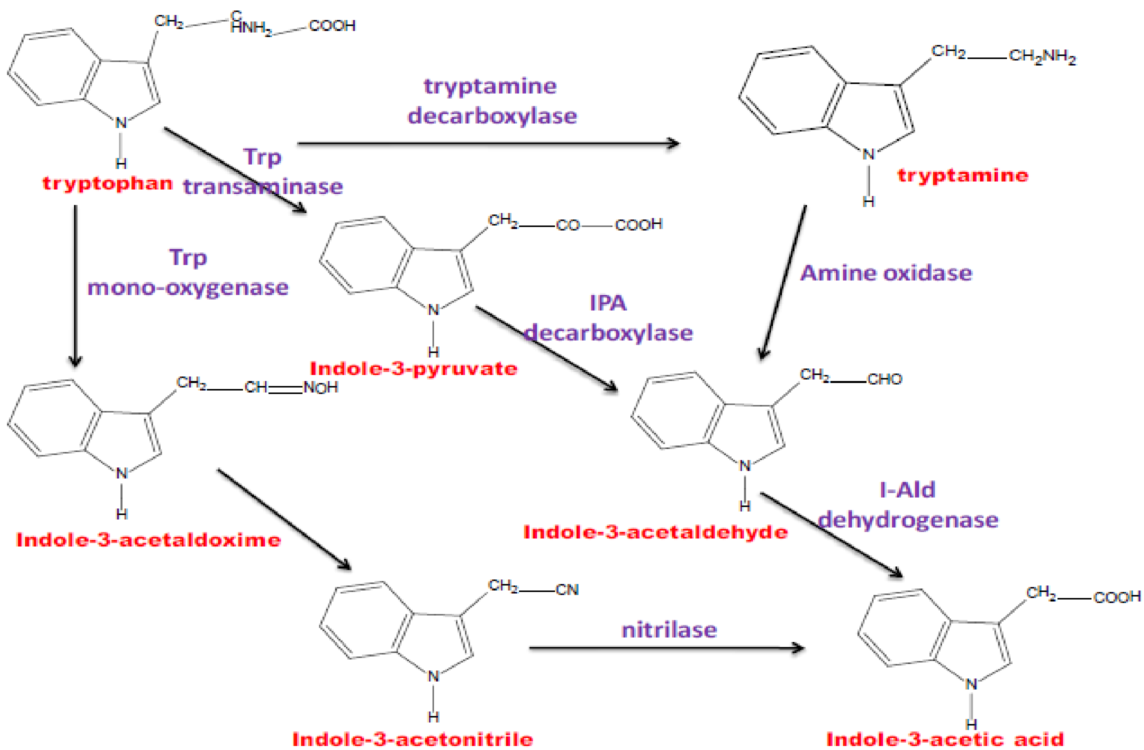
1.5 Biosynthesis of auxin (IAA) in plants

Thimann (1935) found that an amino acid, **tryptophan** is converted into **Indole 3 acetic acid**. Tryptophan is the primary precursor of IAA in plants. IAA can be formed from tryptophan by two different pathways.

1. By **deamination** of tryptophan to form **indole-3-pyruvic acid** followed by **decarboxylation** to from indole-3-acetaldehyde. The enzymes involved are:
    - Tryptophan deamination **deaminase** enzymes
    - Indole pyruvate **decarboxylase**.

2. By **decarboxylation** of tryptophan to form **tryptamine** followed by **deamination** to form indole-3-acetaldehyde and the enzymes involved are:
    - Tryptophan decarboxylase
    - Tryptamine oxidase.

   Indole 3-acetaldehyde can readily be **oxidized** to indole 3-acetic acid (**IAA**) in the presence of indole 3-acetaldehyde dehydrogenase.



1.6 Destruction / Inactivation of auxin in plants

- Auxin is destructed by the enzyme **IAA oxidase** in the presence of O_2 by oxidation. IAA Oxidase

$$IAA + H_2O_2 + O_2 \xrightarrow{\text{IAA Oxidase}} \text{3-methylene oxindole} + H_2O + CO_2$$

- Rapid inactivation may also occur by **irradiation** with **x-rays** and **gamma rays**.
- **UV light** also **reduces** auxin **levels** in plants. Inactivation or decomposition of IAA by light has been called as **photo oxidation**.

1.7 Mechanism of Action

IAA ***increases the plasticity of cell walls*** so that the cells **stretch easily** in response to **turgor pressure**. It has been suggested that IAA acts upon **DNA** to influence the **production of mRNA**. The ***mRNA codes for specific enzymes responsible for expansion of cell walls***.
Recent evidences indicate that IAA increases ***oxidative phosphorylation*** in **respiration** and enhanced **oxygen uptake**. The growth stimulation might be due to **increased energy supply** and it is also demonstrated that auxin induces ***production of ethylene*** in plants.

1.8 Physiological effects of auxin

1. Cell division and elongation

The primary physiological effects of auxin are **cell division** and **cell elongation** in the shoots. It is important in the **secondary growth** of stem and **differentiation** of xylem and phloem tissues.

- The **cell elongation** occurs only in the presence of auxin and the **rate** of elongation is directly proportional to the **amount of auxin** supplied, given no other factors are limiting. However, **relatively high** concentrations of auxin show **inhibitory** effects on this phase of growth.
- Auxin promotes the elongation of **roots** at its **low concentrations**, the growth of roots is retarded at higher concentrations.
- **Flowers** need **higher concentration** of auxin for their growth.
- Auxin also induces the **elongation of coleoptiles** and **stems** by cell enlargement.
- Auxins are responsible for the elongation of petiole, mid rib and major lateral veins of the leaves.
- Hence, adenine aids in enlargement in detached leaves of radish and pea. Similarly, coumarin has been shown to promote expansion of leaves in some plants.

2. Cambial activity:

Activity of Vascular Cambium: Two types of mitotic divisions characterize an active cambium:

1. **Periclinal:** as a result of periclinal divisions new cells of secondary xylem and-phloem are produced and
2. **Anticlinal**: Anticlinal divisions give rise to new cambial initials.

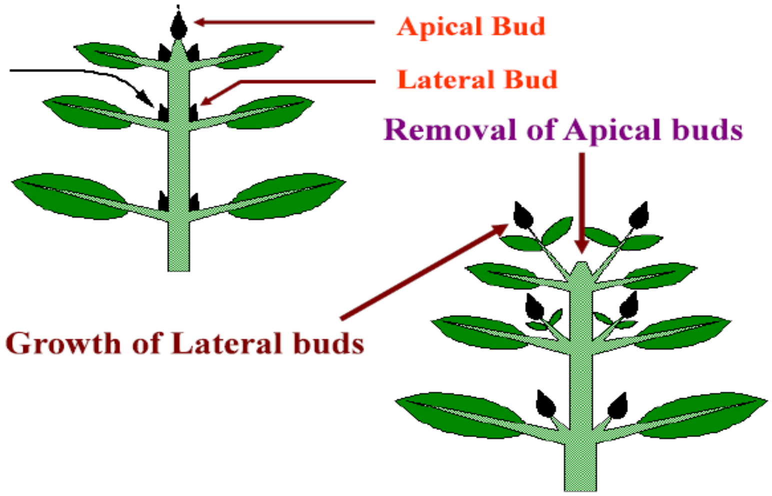
The cambial activity is related to **rainfall** and **temperature** in tropical and temperate zone respectively. In tropical zones the vascular cambium of some species is continually active throughout the entire life. In temperate zones the vascular cambium remains dormant in winter. It activates in spring and produces secondary vascular tissue.
Activation/reactivation of cambial activity followed by a **period of dormancy** occur over the entire life of a plant. It leads to the formation of **growth rings** that reveal approximate **age** of plant.

- During the spring season, the trees manifest growth by developing buds that later on open and elongation of young stems take place.
- Auxin activates this resumed growth by cambial cells.
- The growth moves basipetally in the stems from developing buds.

3. **Apical dominance**

Apical dominance is the major function of auxin. The growth of lateral buds is suppressed until apical bud is present in the plants. This inhibitory effect of terminal bud upon the growth of lateral buds is termed as apical dominance. Skoog and Thimann (1934) first reported the relation of apical dominance with the auxin supply:

- When agar block containing auxin b or IAA was kept on the decapitated shoot of broad bean (*Vicia faba*), the lateral buds, as might be expected, resulted in the usual suppression of growth.
- But when the same decapitated shoot was re-headed with an agar block containing no auxin, these lateral buds resumed growth.
- When **NAA** was used **as auxin** in **field-grown tobacco plants**, similar results were obtained.



Evidence of apical dominance has been practically used in **solving the potato storage** problem:

Potatoes, stored for some time, **sprout** and become **sweet in taste**, causing the grower to **lose financially** as its consumers hate the sweet taste.

But by inhibiting the growth of buds or 'eyes' by **spraying potatoes with auxins** such as indole butyric acid (IBA) and NAA, sprouting (or in other words, prolonging dormancy) can stop sprouting; the effect lasts for as long as 3 years.

4. **Root initiation**

In contrast to stem, the **higher concentration** of auxin **inhibits** the elongation of roots but the number of **lateral roots** is considerably **increased** i.e., higher concentration of auxin induces more lateral branch roots. Application of IAA in **lanolin paste** (lanolin is a soft fat prepared from wool and is good solvent for auxin) to the cut end of a young stem results in an early and extensive rooting. This fact is of great practical importance and has been widely utilized to promote root formation in economically **useful plants which are propagated by cuttings.**

IAA, NAA, 2,4-D, naphthalene acetamide (NAd) etc are the auxins most widely used for this function.

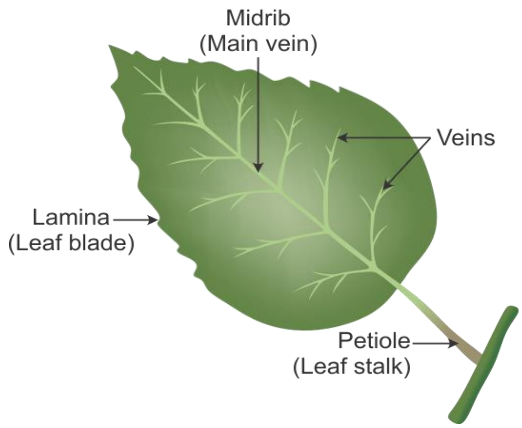
5. **Delay (or inhibition) of abscission of leaves:**

Natural auxins **prevent** the formation of **abscission layer** which may otherwise result in the **fall of leaves**, **flowers** and **fruits**.

By adding auxins on the surface of the lamina or on the cut surface of **a debladed petiole**, abscission of the leaves may be delayed or hindered. Laibach (1933), who demonstrated that the **extract of orchid** pollinia is capable of **preventing leaf dropping**, first noted the regulating actions of auxins on abscission.

Since then, sufficient work in this direction has been carried out. The delaying effect of IAA on the abscission of different plant organs has been shown conclusively by Addicott and Lynch (1955):

- As for the abscission process, it has been proposed that the basipetal migration of a hormone from the blade to the base of the petiole retards the leaf drop.
- Leaf blade removal removes the hormone supply to the abscission zone and thus causes the drop of the leaf.



6. **Callus formation and galls:**

Besides cell elongation, auxin may also be active in **cell division**. In many **tissue cultures**, where the callus growth is quite normal, the continued growth of such callus takes place only after the addition of auxin.

- When **1%** IAA in lanolin paste is applied to a **de-bladed petiole** of a bean plant, **prolific division of parenchyma** cells occurs.
- A **swelling or callus tissue** is formed at the **point** of application of auxin.
- The **amount of callus** tissue formed is directly **proportional** to the **concentration of IAA** applied.

7. **Eradication of weeds**

Some synthetic auxins especially **2, 4- D** and **2, 4, 5-T** are useful in eradication of weeds at higher concentrations.

8. **Flowering and sex expression**

Auxins generally **inhibit** **flowering** **but** in **pine apple and lettuce** it **promotes** uniform **flowering**.

9. **Fruiting:**

Auxins play significant role in fruiting by altering it in one of the following ways:

1. **Fruit setting:**

The **changes in the ovary** leading to the development of the fruit is termed as fruit set.

These changes are generally induced after pollination and fertilization. The development of fruit without fertilization is termed as **parthenocarpy.** It is a common characteristic in plants and hence occurs frequently.

The parthenocarpy can be induced artificially by the aid of auxin. For example, Yasuda (1934) demonstrated it by application of pollen extracts to cucumber flowers. It was also observed that ovaries of many plants (orange, lemon, grape, banana, tomato etc.) could be induced to develop into seedless fruits by application of IAA in lanolin paste to their stigmas.

The various other auxins used for parthenocarpy are IPA, IBA, α-NAA, phenoxyacetic acid (POA), α- naphthoxyacetic acid (NOA) etc.

2. **Fruit thinning:**

The trees, in many cases, bear a large number of fruits. It leads to the inability of the trees to grow an average number of new flower buds. Therefore, such trees must grow fruit either in alternate years (alternate bearing) or if yearly, the number of fruits is significantly reduced.

Clearly, these trees need thinning. For the first time, fruit thinning was achieved in apples when naphthalene acetic acid (NAA) added to flowers failed to set the fruits and actually caused a decrease in the set of fruits. It is interesting to note that the only effective auxin that induces fruit thinning seems to be naphthalene acetic acid.

However, a-2,4,5-trichlorophenoxyacetic acid for thinning of pears and chlorophenoxyacetic acid for thinning of grapes are other examples of auxins used for fruit thinning.

3. **Control of premature fruit dropping:**

The development of an abscission sheet causes the falling of unripe fruits in many fruit trees causes significant losses in yield to the gardeners. In several cases, such as apples, the problem has now been successfully overcome by the application of auxins; Auxins prevent the formation of the abscission layer and thus check the drop of the fruits before harvesting.

With 2,4-D and 2,4,5-trichlorophenoxyacetic acid as auxins, regulation has also been induced in citrus fruits (like oranges and lemons).

4. **Improving the quality of fruits:**

The different processes such as coloring, softening, sweetening and ripening are all involved in improving the fruit 's quality.

In apples, where the use of 2,4, 5-trichlorophenoxyacetic acid has significantly increased red pigments, the auxin effects on fruit colouration are most noticeable.

2,4-D accelerated the ripening process when added to bananas as the auxin stimulates the conversion of starch into sugars.

Sugar accumulation has been reported in sugarcane by injecting 2,4-D, IBA or maleic hydrazide.

10. Respiration

Auxins enhances the respiration process. It was first identified by James Bonner in 1953.

Auxin **stimulates respiration** and there is a correlation between auxin induced growth and respiration. Auxin may increase the rate of respiration indirectly through **increased supply of ADP** by rapidly utilizing **ATP** in the expanding cells.

11. Increased resistance to frost damage:

When parsnip is treated by 2,4,5-T, the tops resist damage by frost.

In apricot fruits, the application of 2,4,5-T before the onset of frost caused less damage than the untreated fruits.

12. Great weapon of war:

When auxins are applied in higher concentrations on enemy crop fields by means of air, it causes devastation of land and form the basis for biological warfare