Review Article

Connotation of Plant Growth Regulators on Seed Germination

ABSTRACT:

Seed germination involves the complex process. Initial requirements for seed germination are suitable temperature (depends on seed type), moisture and oxygen. Endosperm contains food and some hormones for growth initiation. Endogenous and exogenous hormones can play vital role for germination and early growth of seedlings. Plant growth activators are helpful to increase the germination of seeds and retardants inhibit the growth along with the germination process and may induce the dormancy. Therefore growth inhibitors can be used to store seeds for longer time. Many exogenous chemicals are used to control the growth process of seeds and plants. These hormones and chemicals are useful in determination of seed chemistry and can predict its physiology.

KEY WORDS:

growth, inhibitors, exogenous, endogenous, Auxins, Anti-auxins, activators, inhibitors

INTRODUCTION:

Seed is in fact a fertilized egg or a zygote with food reserve in the form of endosperm and the safe cover called seed coat or testa (Figure A). Seeds germination is the growth of zygote to the development of embryo that results in the formation of the seedling on availability of several appropriate conditions. The germination proceeds through three phases, first of all water uptake by seeds (imbibition); activation of food stored in endosperm and finally reactivation of metabolism that results in radical protrusion. It is also a process for activation of metabolic activities inside the seed to grow and form the plumule.

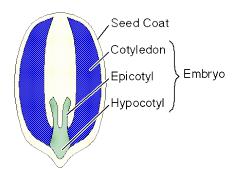


Figure A: Seed Structure [1]

At suitable temperature, oxygen and moisture availability, the seed begins to germinate and the embryo grows into a seedlings. These seedlings require water, minerals along with oxygen for its further growth to plantlets. Along with these appropriate external factors seed need specific hormones for their growth and development. It also involves numerous physiological, morphological, and biochemical changes upon favorable conditions, which are regulated by endogenous and exogenous factors. One of the important internal components affecting germination is hormones. These hormones are called as phytohormones and used to play a vital role in plant growth regulations [2].

Following are different factors involved in the diverse stages of germination process carried out in controlled environment with the help of growth hormones:

1. Imbibition:

The imbibition phase starts with the absorption of water into the seed. This water is taken inside the seed through pores, cracks, and spaces in the seed cover, and is absorbed by the seed tissues. This water absorbed by the dry seed, involves transfer of water by cell wall and macromolecules of endosperm, i.e., proteins and polysaccharides. These water molecules show retention inside the seed by electrostatic forces like hydrogen bonding (Figure B).

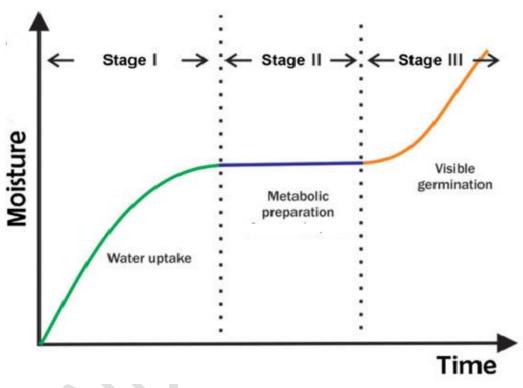


Figure B: Germination of Seed [3]

2. Temperature:

Different seeds show germination at varied temperatures. Most of seeds germinate between 25-30 0 C, Some seeds require high temperature higher than normal (30-40 0 C, summer crops) and some requires very low even up to 5 0 C (winter crops). Temperature below its standard required temperature retards or inhibits the embryonic activities or germination process whereas high temperature terminates hormones production and cell division. High temperature promotes abscisic acid production and ultimately dormancy is attained [4].

Temperature plays a key role in seed germination, and it affects the cell energy status and enzymes'activity (e.g., lipase, alanine aminotransferase, aspartate aminotransferase, and especially ribonuclease) takes place as the temperature increases and the ATP content rises. The rate of protein synthesis decreases with the increase of temperature. Since germination involves several stages, each has its cardinal temperature scale; the temperature response can differ during the germination period because of its complexity [5]. The temperature reaction of a seed depends on variety, seed quality, the length of time after harvest, and some other factors. The germination process requires different temperature changes depending on rate of respiration and sugar metabolism. Temperature role in specific in different seed types, it not only determines the germination duration, but also controls water to the initiation of germination [6].

3. Oxygen:

As germination starts in seed, it respires vigorously to consume food and release the energy required for its growth. Oxygen deficiency affects or may abort the seed germination. Oxygen is used in respiration and speed up the metabolism reactivation during seed imbibition, Seed coat or testa inhibit the respiration, hence imbibations leads to the softening of seed coat and hydrolysis of endosperm to generate energy in the form of ATP. Due to imbibition there is enzymatic oxidation of phenolic compounds by polyphenol oxidases (catechol oxidase and laccase) and peroxidases. The key role of oxygen in the molecular networks regulating seed germination and dormancy through the hormonal activation of ethylene, gibberellins and the emerging role of mitochondria in respiration of embryo [7].

4. Phytohormones are plant hormones present in very low concentration and can control the different developmental condition ranging from embryo to well-developed plants. Synthetic plant hormones are exogenously applied for controlled crop production. These exogenous and endogenous plant hormones control all growth and development activities like cell division, enlargement, flowering, seed formation, dormancy and abscission. Phytohormones are of different types based on their structure and functions. There are different classes of such hormones.

- i. Auxins
- ii. Gibberellins
- iii. Abscisic acid
- iv. Cytokinins
- v. Brassinosteroids
- vi. Jasmonates
- vii. Ethylene

The seed cotyledons treated with different concentrations of auxins or with varying concentration of diverse cytokinins show increase in urease activity, compared to the control. The optimal effects can be observed for each of 500 µmol of auxins and 300 µmol of cytokinins treatments. A gradual increase in urease activity is detected in cotyledons treated with various concentrations (0.2-1.0 mM) of brassinosteroids, in comparison to control. These hormones play important role in plant physiology and are responsible for growth and development [8]. These are of different structures and may perform similar functions. Based on their action, plant hormones are classified as:

- Seed growth activators
- Seed growth Inhibitors

Auxin Hormone:

Auxins play an important role in growth and control the different cycles of development. Auxins play an important role in seed development. It regulates the growth of an embryo, development of endosperm and rapture of seed coat. Auxins level varies in growing seeds. In various stages theses are directly involved in the growth of fertilized seed after the availability of moderate environmental conditions. Auxin releases as endosperm development take place after seed germination. Auxin means "to grow". They are widely used in agricultural and horticultural practices. They are found in growing apices of roots and stems and then control all plantlets and hence is responsible for plant growth.

Natural Auxins: there are five different types of auxins naturally occurring in plants, like Indole-3-acetic acid (IAA, 1), Indole butyric acid (IBA), phenyl acetic acid, indole Propanoic acid and chloroindole-3-acetic acid. Auxins are also known as indoles. Indoles are benzene ring fused with pyrrole, have a general formula C₈H₇N. Indole-3-acetic acids, indole-3-butyric acid and its derivatives are also known as auxins, found in plants as plant growth regulators. The auxins are responsible for cell division and also responsible for geotropism, phototropism and hydrotropism. Auxins are present in the shoot buds and induce shoot apical dominance [9].Synthetic: 2,4-D (2,4-Dichlorophenoxyacetic acid), NAA (Naphthalene acetic acid) are extensively used as pro-auxins to improve the growth of plants and for rapid growth.

Mechanism of Action:

Auxins play important role in embryo development after seed imbibations. The concentration of auxin controls the differentiation of the embryo into different organs of the plant, including the shoot apex, primary leaves, cotyledon(s), stem, and root.

The auxins are transported by polar transport system through parenchyma cells. The cytoplasms of parenchyma cells are neutral (pH=7) where auxins releases proton and becomes anion (IAA⁻). It cannot pass through hydrophobic portion of the plasma membrane as an anion, but it does pass through special auxin efflux transporters called PIN proteins. The adjacent cells to cytoplasm (apoplast) are acidic (pH=5) in nature, IAA⁻ enters the acidic environment of the apoplast, it is again protonated to form IAAH that is neutral.

This uncharged molecule can then pass through the plasma membrane of adjacent cells through diffusion or via influx transporters. Once it enters the cytoplasm, it loses proton to form IAA⁻ anion. PIN proteins are found around the cell and direct the flow of auxin [10].

Auxin effects are some on the cell to initiates the patterns of gene expression and movement of ions in and out of the cell and reduction in the redistribution of PIN proteins. Inside the cell auxin increases the longitudinal cell size and ultimately rate of cell division increases. The auxin may bind to a cell-surface receptor or called as Auxin-binding protein (Figure C).

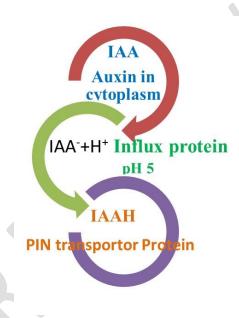


Figure C: Mechanism of transportation of Auxins [10]

Functions:

- It carries the fertilization of ovum by sperm and transformation in to zygote.
- It carries the transformation of zygote in to the embryo and initiates germination in collaboration with gibberellins (Figure D).
- It induces the development of seed coat after fertilization and development of embryo,
- It plays role of differentiation and division of endosperm and therefore proceed to further stages.
- There is storage of auxins in developed embryo.
- It is responsible for cell elongation of seeds endosperm and ultimately growth of plumule and radicle.
- Induces parthenocarpy i.e. development of fruit without fertilization e.g. in tomatoes
- It is responsible for fixing of newly formed leaves in plumule and keeps them green for longer period.
- Useful in stem cuttings and grafting where it initiates rooting
- Promotes flowering e.g. in pineapple
- Auxins in combination with abscisic acid induces dormancy in seeds (Figure E).
- 2,4-D is widely used as a herbicide to kill undesirable weeds of dicot plants without affecting monocot plants.when It is sprayed on leaves, it gets absorbed through the leaves and is transferred to the meristems of the plant. Unrestrained herbs growth is controlled by curl-over of week stem, leaf sarcastic effect that turns over the plant death [11].



Figure D: Seed Germination by Auxins

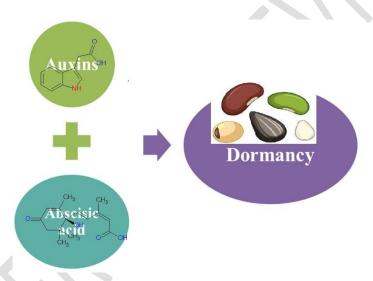


Figure E: Seed Dormancy by Auxins

Gibberellins:

Gibberellins (2) are of wide variety, more than 100 gibberellins (GA₁, GA₂, GA₃.....) are known found in seeds and in plants also. These are acidic in nature due to presence of –COOH group. It is used for pre-treatment of seeds as they produce amylase, results in germination. Gibberellins are the plant regulators, involves in regulation of the growth and influencing different developmental processes which includes stem elongation and germination and enzyme induction [12].

As water absorption takes place inside the seed, imbibition starts. The hormone signal in seeds like Gibberellic acid activates the DNA in the cells; a gene for amylase gets activated results in the production of the amylase enzyme inside the cells. The amylase starts working in the endosperm area. There amylase breaks down starch into sugar which is transported to the hypocotyle or embryo of seed. The sugar content increases the respiration rate in the embryo for its growth. The radicle protrusion from the seed coat results the initiation of germination (Figure D).

Mechanism of action:

Gibberellins are transported in plants in non-polar way. It is translocated in bounded form as gibberellins-glycoside linkage. In suitable conditions such as light, temperature and water seed absorbs water and gets imbibe. It stimulates the production of gibberellins

Gibberellin transfers to aleurone layer of endospem to synthesize amylase. It increases the mRNA in order to code the amylase production. The amylase acts on the starch molecules in the endosperm, producing soluble maltose or sugar molecules. The maltose is then turned into glucose and is available as a food for developing embryo (Figure F). This glucose is used by the embryo during the respiration process to provide the energy needed for its growth [13].

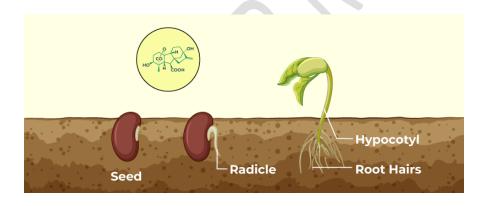


Figure F: Seed Growth by Gibberellins [14]

Functions:

- It delays senescence.
- It induces the formation of hydrolytic enzymes such as proteases, lipase and amylase in the endosperm of germinating grains, barley seeds and others.
- It breaks seed dormancy and initiates seed germination.
- Seeds pre-treated with gibberellins shows increased germination rate
- A pretreated seed also shows increased radicle and hypocotyl length.

Cytokinins:

Cytokinins (3) primarily play an important role in cytokinesis process. Cytokinins are naturally synthesized in the plants where rapid cell division occur e.g. root apices, shoot buds, young fruits. There are two types of cytokinins: adenine-type cytokinins such as kinetine, zeatine (corn kernels, coconut milk) and benzylaminopurine, and phenylurea-type cytokinins like diphenyl urea and thidiazuron (TDZ). Most adenine-type cytokinins are synthesized in roots, tissues and cambium phenylurea cytokinins are not found in plants these are usually synthetic. Cytokinins are transported inside plants through xylem [15].

Mechanism of action:

Cytokinins bind to receptors found inside the endoplasmic reticulum membrane, triggering the activation of histidine kinases. These activated histidine kinases is used to convert the phosphate groups to histidine phosphotransfer proteins. Phosphorylated AHPs, in turn, transfer the phosphate group to type-B response regulators (RRs), it enters the nucleus and regulate the expression of specific target genes involved in cell division, shoot growth, and apical dominance. This regulatory process ultimately leads to increased cell division and lateral shoot growth of developing embryo [16]. It works in collaboration with auxins that are involved in sugar formation and strigolactone and hence play key role in the development of embryo (Figure G).

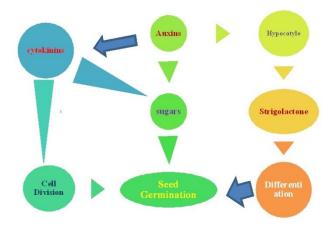


Figure G: Cytokinins mode of action with Auxins and Strigolactone [16]

Functions:

- It promotes the germination by increasing the action of auxins.
- It also inhibits the action of abscisic acid thus breaks dormancy.
- It promotes lateral and adventitious shoot growth and used to initiate shoot growth in culture
- It reduces the apical dominance by controlling the auxins.
- Stimulate the formation of chloroplast in leaves
- Promotes nutrient mobilization

Abscisic Acid:

Abscisic acid (ABA, 4) is sesquiterepenoid (C_{15}) plant hormone that regulates the plant growth, development, stress responses and reduce dormancy. It is synthesized from carotenoids (C_{40}) via oxidative cleavage. ABA catabolism is initiated by several modifications such as oxidation and conjugation. Movement of ABA inside plant is an important for the immediate responses to drought stress. It is also called stress hormone as it increases the tolerance of plant against drought.

It is a growth-inhibiting phytohormone and works in collaboration with Gibberllins. It inhibits plant metabolism and regulates abscission and dormancy.

Mechanism of action:

Abscisic acid binds with specific receptors associated with G-proteins located on the plasma membrane of plant cells. ABA binding with G-proteins initiates the production of secondary messengers like calcium ions and atomic oxygen. The secondary messengers are responsible for protein phosphorylation events, leading to modifications in gene expression. The altered gene expression activates stress-responsive genes and triggers the closure of stomata, enabling plants to conserve water during drought and stress conditions.

Functions:

- Induces abscission of leaves and fruits
- Inhibits the seed germination
- Induces senescence in leaves
- During drought it accelerates dormancy in seeds that is useful for storage purpose
- Stimulates closure of stomata to prevent transpiration under water stress

Ethylene

Ethylene (5) occurs in gaseous form. It is synthesized in the ripening fruits and tissues undergoing senescence. It regulates many physiological processes and one of the most widely used hormones in agriculture. Ethylene supports seed germination along with seedling growth through increased hypocotyl elongation. Ethylene inhibits primary root growth by reducing cell expansion. In this way it can acts as a growth promoter as well as an inhibitor.

Mechanism of action:

Ethylene is a gaseous hormone that diffuses through the cell membrane and binds to receptors in the endoplasmic reticulum (ER) of plant cells. The binding of ethylene to its receptors triggers the activation of the transcription factor EIN₃. EIN₃ express the genes associated with the "constitutive triple response," causing the plant to display a unique growth pattern characterized by a thickened and shortened stem, radial swelling, and horizontal growth.

Functions:

- It regulates the seed dormancy by breaking the seed coat and therefore ends dormancy
- It helps the ripening of fruits
- It stimulates rapid elongation of petioles and internodes
- It promotes the abscission of leaves and flowers
- It reduces root growth or radicle growth in seeds and increases root hair formation in early plantlets thereby increasing the absorption surface.

Brassinosteroids:

Brassinosteroids (6) are a class of polyhydroxysteroids that have been recognized as a class of phytohormones and may have utility as anticancer drugs for treating endocrine-responsive cancer cells by inducing apoptosis and inhibiting cancerous growth. It promotes stem elongation and cell divisions, and the biologically active molecule in humans.

Mechanism of action:

Brassinosteroids initiate the binding to receptors on the cell surface and cause triggering the activation of a receptor complex. It in turn, phosphorylates and activates the Kinases. The phosphorylates and activates the transcription factor of genes, that regulates the expression of cell elongation, cell division, and stress responses, leading to enhanced growth and improved stress tolerance in plants.

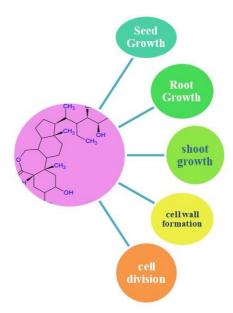


Figure H: Function of Brassinosteroid in seed growth and germination

Functions:

- 1. Brassinosteroid (BR) hormones play a role in promoting germination.
- 2. Seed dormancy and germination are regulated by the plant hormones, abscisic acid (ABA) and gibberellin (GA) both.
- Hormones act antagonistically with each other. ABA induces seed dormancy in maturing embryos and inhibits germination of seeds. GA breaks seed dormancy and promotes germination.
- 4. BR stimulates germination and raises the possibility that it is required for normal germination. BR mutants exhibit a germination phenotype in the presence of ABA. Thus, the BR signal is needed to overcome inhibition of germination by ABA (Figure F).

Jasmonates:

Jasmonates (Jas, 7) are important plant hormones that mediate stress responses, and prone to have inhibitory effects on seed germination, thus act as plant growth inhibitors or growth retardants.

Functions:

- By studying mutants overexpressing JA, one of the earliest discoveries made was that JA inhibits root growth.
- JA plays many roles in flower development. The same genes promoting male and female fertility.

- JA and methyl jasmonates inhibit the germination of non-dormant seeds and stimulate the germination of dormant seeds.
- Jasmonates treated seeds are resistant to germination and such seeds after germination show good growth and saves plant from the attack of spider mites, caterpillars, aphids, and fungal pathogen.
- Jasmonates can be used as signal to close the traps and to control the release of enzymes
 and nutrient transporters which are used in plant digestion. However, not all carnivorous
 plants rely on the jasmonate pathway in the same way.

5. EXOGENOUS GROWTH REGULATORS

Natural PGRs are usually in small amounts to control essential functions inside the plants. Occasionally there is need of some exogenous assistance in agriculture through plant extraction or synthetic plant growth regulators to assist some metabolic or growth functions in seeds or plantlets. Furthermore, the production of natural PGRs through biosynthesis is restricted due to technical issues. There are many synthesized compounds that are phytohormone analogues to natural plant growth regulators. These compounds can easily be synthesized, but their mode of action need to be studied for further structural optimization and mechanism exploration.

- **Urea and thiourea** derivatives possess growth inhibiting properties and increase salt tolerance in wheat and are useful to save plants from chlorophyll degradation of chlorophyll in seeds. It may cause dormancy and saves seeds from germination [17].
- Synthesized **pyrazole derivatives** can cause the seedlings growth and impede root growth. It is analogue to the natural phytohormone. The hypocotyl length of seedlings can be increased after treatment with pyrazole derivatives. The comparative analysis of plant growth regulating the activity of new synthetic low molecular weight. In this regard, the elaboration of new effective ecologically safe plant growth regulators on the base of synthesis. The low molecular weight heterocyclic compounds like derivatives of pyrimidine, pyrazole, and oxazole are extensively used in the agriculture as plant growth regulators, herbicides, fungicides and antibacterial agents [18].

- Pesticides are highly toxic chemicals used as herbicide in crops. There are four commonly used pesticides such as, emamectin benzoate, alpha-cypermethrin, lambda-cyhalothrin and imidacloprid can inhibit the germination of seeds. The seed germination get slow down if these pesticides are already present in the soil and this effect is more pronounced when seeds are sown in the soil containing such hazardous chemicals [19]. The synthesized dichloroacetamides are proved to be a novel PGRs that basically effect the growth of roots [20].
- The derivatives of dehydroamino acids 2,3-dehydroaspartic acid dimethyl ester, potassium salt of 2-amino-3-methoxycarbonylacrylic acid and 1-methyl-3-methylamino-maleimide show their growth-regulating properties. Dehydroamino acids 2,3-dehydroaspartic acid dimethyl ester shows the most stimulating activity on all test.
- Series of **alkyl silatranes** as PGRs. These compounds are growth accelerators of wheat and maize. In maize, 3-aminopropyl silatranes gave the best results at 100 μmol L⁻¹, whereas for wheat 3-chloropropyl silatrane is good growth accelerators at 200 μmol L⁻¹ [21].
 - **Urea derivatives** can exhibit extraordinary inhibition activity on root growth the inhibition activity is higher than that of the commercially available herbicide clopyralid. 1-phenethyl-3- (3-(trifluoromethyl) phenyl) urea, potent inhibition activity on root growth of Arabidopsis [22].
 - **Ureas, carbamates and oxamates** exist in nature and as synthetic compounds. These exhibit a wide spectrum of biological activity. A number of compounds with core imidazolidin-2-one and aryl moieties as urea and carbamate derivatives are also proved as plant growth regulatory activity especially show influence on and development of seeds [23, 24].
 - **Aspartic acids:** The derivatives of dehydroamino acids 2,3-dehydroaspartic acid, its dimethyl ester, potassium salt of 2-amino-3-methoxycarbonylacrylic acid and 1-methyl-3-methylamino-maleimide are synthetic plant growth-regulators. Dehydroamino acids 2,3-dehydroaspartic acid dimethyl ester possess the growth activating and is effective on different crops along with environment friendly. These can be used in the form of

microcapsule formulations through which PGRs can release as diffusion. It may be helpful in future as favorable agro-formulations with a constant and targeted release for plant growth regulation [25].

- Bojack et al. have synthesized 2,3-dihydro-1-benzofuran-4-carboxylates and quinolones. It can regulate the affinity of seed to compete against the drought and cold stress in wheat, corns and canola [26].
- Indazoles: Indazoles (8) are organic heterocyclic compounds in which benzene fused with pyrazole. It is rarely found in nature for growth regulation or particularly germination process. It is found in Nigella Sativa or black cumin is only source of indazole alkaloid. Synthesized indazoles are also found to be plant growth regulators by inhibiting the germination of seeds. Derivatives 3-aryl-1*H*-indazoles show its effects on seed germination and early growth. The arylindazoles are growth inhibitors of root and shoot lengths of wheat and sorghum, especially at a high concentration (100 ppm). At lower concentrations growth inhibition seems to be less pronounced. Seed germination and early growth of plantlets also depends on the nature of substitution on the aryl group [27].

• Indazole-3-acetic acids: are found to be depressant of seed germination hence can be successfully used for crop seeds storage in extremely hot weather of Pakistan. In wheat effect of these compounds on early growth is acceleration in all concentrations. In sorghum at high concentrations these compounds were proved to have inhibitory effect more pronounced on root and shoot growth while at low concentration effect is negligible in comparison to control. 3-Arylindazole-1-acetic acids are supposed to delay the germination of seeds simply by lowering the metabolic process during the imbibitions but early growth of seed show somewhat acceleration [28]

• Coumarins and Coumarin-3-acetic acids: Coumarin (1-benzopyran-2-one, 9) are the secondary metabolites in some plants like legumes, clover, medicinal plants possess variety of biological functions i.e. anti-oxidants, anti-inflammatory, anti-coagulant, antibacterial, antifungal and other pharmacological actions. Coumarins are growth retardants or can suppress the function of auxins while coumarin-3-acetic acids although are not synthesized in plants, its growth retardant effect is more pronounced and are called as anti-auxins [29]. Synthesized coumarins are growth retardants and inhibit the germination process along with early growth of plantlets.

Coumarin-3-acetic acids: Number of synthesized coumarin-3-acetic acids is proved to be growth inhibitors and inhibit the germination process. These are used to induce dormancy. Coumarin-acetic acids are more potent growth inhibitors in comparison to coumarins itself. By experiments it was observed be more germination inhibitor in comparison to abscisic acid (Figure I). Such compounds can be used as herbicide and can induce dormancy for seed storage.





Figure I: Role of Abscisic Acid and Indole butyric Acid (IBA) on seed germination [28]

CONCLUSION:

Plant growth regulators play a vital role not only in seed germination but also in early growth of plantlets. Auxins play an important role in seed development, regulates the growth of an embryo, development of endosperm and rapture of seed coat. The concentration of auxin controls the differentiation of the embryo into different organs of the plant, including the shoot apex, primary leaves, cotyledon(s), stem, and root. Gibberellins are used for pretreatment of seeds to produce amylase and results in germination. After imbibations, the sugar content increases the respiration rate in the embryo for its growth. The radicle protrusion from the seed coat results the initiation of germination. It involves in regulation of the growth and influencing different developmental processes which includes stem elongation and germination and enzyme induction.

Cytokinins are naturally synthesized in the plants where rapid cell division occur e.g. root apices, shoot buds, young fruits. Abscisic acid is a growth-inhibiting phytohormone and works in collaboration with Gibberllins. It inhibits plant metabolism and regulates abscission and dormancy. Ethylene supports seed germination along with seedling growth through increased hypocotyl elongation. Brassinosteroids promotes stem elongation and cell divisions. Jasmonates mediate stress responses, and prone to have inhibitory effects on seed germination, thus act as plant growth inhibitors or growth retardants. Some of exogenous compounds are used in agriculture as plant growth regulators to assist some metabolic or growth functions in seeds or plantlets. There may be a restricted production of natural PGRs through biosynthesis due to some technical issues. There are many synthesized compounds such as urea, thiourea, pyrazole, aspartic acids, quinolones, indazoles, coumarins and coumarin-3-acetic acids derivatives that are phytohormone analogues to natural plant growth regulators. These compounds are used to promote and retard the growth and metabolic process.

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