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Understanding plant growth regulators, their interplay: For nursery establishment in fruits

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Abstract

The term plant growth regulator (PGR) includes naturally occurring plant growth substances or phytohormones, as well as synthetic compounds which mostly are chemical analogs, materials that alter hormone levels (hormone releasing agents or synthesis inhibitors) and materials that alter hormone sensitivity. The concentrations of hormones required for plant responses are very low (10^{-6} to 10^{-5} mol/l). Because of these low concentrations, it has been very difficult to study plant hormones and only since the late 1970's have scientists been able to start piercing together their effects and relationships to plant physiology. Since the 1940's both natural and synthetic growth regulators have been used in agriculture and horticulture with increasing incidence to modify crop plants by controlling plant developmental processes, from germination to post harvest preservation. The concentrations, time and method of applications play an important role in determining the effect of these chemicals on varied fruit crops. Plant growth regulators have a great use for nursery growers from storage of planting material to branch initiation. As, quality planting material is a paramount concern of the researchers and fruit grower's for better growth and yield of plants. Exogenously applied GA_3 overcomes seed dormancy in several species and promotes germination in some species that normally require cold stratification, light, or after-ripening. Plant bio-regulators helps in formation of good architecture which will enhance better quality production, better utilization of land and are known to develop higher number of branches, higher leaf area and better ratio between tiny roots and skeletal ones.

Keywords: plant growth regulators, propagation, architecture.

Introduction

Unlike animals, plants can manufacture all the chemical substances they need for survival, given only light, water, carbon dioxide and trace elements from the soil and environment. They manufacture all the amino acids, carbohydrates, nucleic acids and other compounds including and among these are hormones. The word Hormone has been derived from the Greek word horman, meaning "to stimulate").

Plant hormones (also known as phyto-hormones) are naturally occurring organic substances that affect growth and development in very low concentrations and whose action may be involved in sites far from their origin. Hormones helps in the formation of flowers, stems, leaves, the shedding of leaves and the development and ripening of fruit. Hormones are crucial to plant growth and lacking them, plants would be mostly a mass of undifferentiated cells (Jain, 2013; Byers, 2000) [7, 2].

Plant growth regulators (PGR's) are organic compounds other than nutrients that we apply on plants to cause any physiological response. The term plant growth regulator (PGR) includes naturally occurring plant growth substances or phytohormones, as well as synthetic compounds which mostly are chemical analogs, materials that alter hormone levels and materials that alter hormone sensitivity. In general plant hormones can be divided into (1) Plant growth promoters (substances which improve the overall health, growth and development of plants) and (2) Growth retardants (group of chemicals which have common physiological effect of reducing stem growth by inhibiting cell division of sub apical meristem). The concentrations of hormones required for plant responses are very low (10^{-6} to 10^{-5} mol/l). Because of these low concentrations, it has been very difficult to study plant hormones and only since the late 1970's have scientists been able to start piercing together their effects and relationships to plant physiology (Byers, 2000) [2]. Plant growth and development is ultimately the function of all essential elements and five important hormones (gibberellins, auxins, cytokinins, abscisic acid and ethylene). Their desirable effect is manifold when sprayed in combination on the plants (Hajam, 2017) [1].

Generalized model for a hormone-triggered signal transduction pathway:

A hormone binds to a definite protein receptor, either embedded in the plasma membrane or in the cytoplasm (depending on the receptor system).

- Binding of the hormone to the receptor causes the protein's conformation to change.
- This stimulates the production of "relay molecules" in the cytoplasm.
- Relay molecules trigger various responses to the original signal.

A given plant hormone may

- Stimulate different responses in different tissues.
- Stimulate different responses at different times of development in the same tissue.
- Affect the activity or production of other hormones directly.
- Give different responses depending on concentration in a given tissue.

The sensitivity of a plant tissue to a given hormone may be altered either by

- Changing the concentration of hormone in the target tissue.
- Altering sensitivity of receptor to that hormone in the target tissue.

The main five hormones

There are five (also called big five) well established categories of “classical” phytohormones, namely:-

- Auxins.
- Gibberellins.
- Cytokinins.
- Abscisic acid.
- Ethylene.

The chemicals are each grouped together into one of these classes based on their structural similarities and on their effects on plant physiology. Each class has positive as well as inhibitory functions and most often work in tandem with each other, with varying ratios of one or more interplaying to affect growth regulation.

More recently, several other compounds that can regulate various activities of plant growth and development have been described, such as oligosaccharins, brassinosteroids, salicylic acid, jasmonates, polyamines, nitric oxide (NO), strigolactones, karrikins etc.

(a) Auxins

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. Auxins were the first class of growth regulators discovered. They affect cell elongation by altering cell wall plasticity. Auxins decrease in light and increase where it is dark. They stimulate cambium 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. Auxins in seeds regulate specific protein synthesis, 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 hefty concentrations; they are most toxic to dicots and less 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-Naphthalene acetic acid (NAA) and Indole-3-butyric acid (IBA), are also commonly applied to stimulate root growth when taking cuttings of plants. The most common auxin found in plants is indole acetic acid (IAA) but other forms occur, and many IAA-like compounds are used commercially because they are more stable than natural IAA, which is readily broken down by plant enzymes, fungi and bacteria. The correlation of auxins and cytokinins in the plants is a constant (A/C = const.).

Effects

- Differentiation of meristem into vascular tissue, promotes leaf formation and arrangement, induces flowering, inhibits leaf abscission, apical dominance, fruit development and ripening (from auxins produced by maturing seeds). Parthenocarpic (seedless) fruit can be induced to ripen with application of IAA.

(b) Gibberellins

Gibberellins include a large array of chemicals that are produced naturally within plants and by fungi. They were first discovered when Japanese researchers, including E. Kurosawa, noticed a chemical produced by *Gibberella fujikuroi* that produced abnormal growth in rice plants. 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 GAs. The GAs are transported to the aleurone layer, which responds by producing enzymes with the purpose of break down stored food reserves within the endosperm, which are utilized by the growing seedling. GAs produce bolting of rosette-forming plants, escalating internodal length. They promote flowering, cellular division, and in seeds growth after germination. Gibberellins also reverse the inhibition of shoot growth and dormancy induced by ABA (abscisic acid).

Effects

Promote growth (especially those plants are genetically dwarf types), bolting and flowering, replace chilling requirements of plants and light requirements, promote seed germination and break dormancy, increase cell elongation and induce maleness.

(c) Cytokinins

Cytokinins are a group of chemicals that influence cell division and shoot formation. They were called ‘kinins’ in the past when the primary cytokinins were isolated from yeast cells. They also help delay senescence or the aging of tissues, are responsible for mediating auxin transport throughout the plant, and affect 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. The correlation of auxins and cytokinins in the plants is a constant (A/C = const.).

Effects
Cell division, shoot initiation, breaking dormancy, promote seed germination, retard senescence, promote hermaphrodite flower. e.g., Grape and induce parthenocarpic fruit.

(d) Ethylene
Ethylene is a gas that forms from the breakdown of methionine. Ethylene has very limited solubility in water 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 by phytochrome in the plant's cells produce a signal for ethylene production to decrease, allowing leaf expansion. Ethylene production prevents cell elongation and causing the stem to swell. 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, sturdier tree trunks and branches. Ethylene affects fruit-ripening, normally, when the seeds are mature, ethylene production enhances and builds-up within the fruit, resulting in a climacteric event just before seed dispersal. The nuclear protein Ethylene insensitive 2 (EIN2) is regulated by ethylene production, and, in turn, regulates other hormones including ABA and stress hormones.

Effects
Apical dominance arrested, stimulates lateral growth, promote abscission of leaves, flowers, and fruits, induction of flowering, helps in fruit ripening, promote rooting, helps in chlorophyll formation, promote seed germination and increase female flowers.

(e) Abscisic acid
Abscisic acid 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 latter compounds were 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.

Effects
Prevents cell division thus suppresses the growth of plants., accelerate flower initiation, senescence and abscission, inhibits root development, promotes bud and seed dormancy, closing of stomata is caused by ABA as stress response (Jain, 2013) [7].

(Greene, and Costa, G. 2010; Santner, *et al.*, 2009; Elfving and Visser, 2006; Byers, 2000) [6, 10, 3, 2].

Other known hormones
Other identified plant growth regulators include:

- **Brassinosteroids-** are a class of polyhydroxy-steroids and represent a group of plant growth regulators. Brassinosteroids have been recognized as a sixth class of plant hormones which stimulate cell elongation and division, gravitropism, resistance to stress and xylem differentiation. They inhibit root growth and leaf abscission. Brassinolide was the first recognized brassinosteroid and was isolated from organic extracts of rapeseed (*Brassica napus*) pollen in 1970.

- **Salicylic acid**- activates genes in some plants that produce chemicals that aid in the defence against pathogenic invaders.

- **Jasmonates**- are produced from fatty acids and seem to promote the production of defence proteins that are used to defend off invading organisms. They are also believed to have a role in seed germination, and affect the storage of protein in seeds, and seem to affect root growth.

- **Plant peptide hormones**- encompass all small secreted peptides that are involved in cell-to-cell signalling. These small peptide hormones play crucial roles in plant growth and development, including defence mechanisms, the control of cell division and expansion, and pollen self-incompatibility.

- **Polyamines**- are strongly basic molecules with low molecular weight. They are essential for plant growth and development and affect the process of mitosis and meiosis.

- **Karrikins-** are the group of plant growth regulators found in the smoke of burning plant material and has the ability to stimulate the germination of seeds.

(Jain, 2013; Greene, 2010; Greene and Costa, 2010) [7, 5, 6]

Marketed plant growth regulators
Plant growth regulators account for only roughly 2.5% of the total trade of products used in plant protection. This limited market potential, enormous costs for development and registration, and the demand for high sales and profitability have created major constraints to the introduction of new plant growth regulators. However, it can be assumed that there are still potential to successfully introduce new products. Plant growth regulators have become an integral part of modern agricultural, horticultural practice. Some of the marketed plant regulators along with their active ingredients and crops in which mostly used is given below in the table.

List of marketed plant growth regulators with active ingredient/s

Active ingredient	Product Name	Crops
2-(1-naphthyl) acetic acid	Planofix, FruiTone, Fruit Fix 200, Fruit Set, Stafast	Apple, Pear, Pineapple
4-indol-3-butyric acid	Seradix	Fruit Crops and Ornamentals
Gibberellins	ProGibb, NovaGib, ProVide	Grape, Pear, Citrus, Potato, Mango, Grape seedless
6-benzyl adenine	MaxCel, GerBA	Apple, Cherry, Grape, Pear, Citrus

6-benzyl adenine/gibberellins	Promalin, Progerbalin	Apple, Plum, Flowers, Ornamentals
Ethephon	Ethrel	Apple, Cherry, Citrus, Cotton, Grape, Maize, Peach, Pineapple, Plum, Prune
Paclobutrazol	Cultar	Litchi, Mango, Peach Plum
Daminozide	B-Nine SP	Flowers, Ornamentals
Prohexadinone-Ca	Apogee	Apple, Pear, Wheat
Brassinolide	Double	Agri. and Horti. Crops

Major uses of plant growth regulators in nurseries of fruit crops:

(1) Use of PGRs in plant propagation

All types of propagation methods are influenced by PGRs which may sexual through seed or asexual through cutting, layering etc. or may be modern tissue culture technique.

(a) Seed

GA significantly accelerates seed germination in many fruit plant species. Gibberellins are used substitution of chilling requirement which remains essential for germination of many fruit seeds. Embryo dormancy is due to the presence of inhibitors such as ABA therefore the lowering the level of ABA in seeds promotes seed germination. Loss of this dormancy is given by a drop in the ABA: GA ratio. The GA is needed to break down the storage reserves for food and release of energy occurs and germination is promoted**.** Gibberellic acid and auxin intensifies an organ ability to function as a nutrient sink (Hajam, 2017) [1] hence may be important for carbohydrate accumulation in the seeds.

Wani *et al.* in 2014 concluded that different concentrations of gibberellic acid (GA_3) endorsed germination percentage and growth of seedlings in apple seed i.e. radical and plumule elongation. In their study, in order to remove dormancy in apple seeds, exogenous GA_3 application has been most successful in breaking dormancy at 500 ppm concentration. It was recommended that the concentration of growth regulators GA_3 500 ppm for 40 hrs favors the increased enzymatic activity that leads to the favorable environment for the seed germination as well as the growth of the radicle and plumule leading to better growth and survival of seedlings.

(b) Cuttings

All types of PGRs have either direct or indirect effect on rooting of cuttings which can be either positive or negative. All types of auxins some cytokinins are used to induce rooting. Generally IAA, IBA, NAA, CPPU etc are used to induce rooting of cuttings and root initiation in layered plants. NAA increase rooting in stools of hard to root rootstocks. GA is found to have antagonistic effect on rooting of cuttings. Generally the cuttings are divided into three types depending upon their age and the auxins required hence vary with the type of cuttings as hard wood cutting require more than 1500 ppm of IBA similarly semi hard wood 500-1500 ppm and soft woodless than 500 ppm of IBA for rooting initiation.

In 2006, Tripathi *et al.* reported that the application of NAA increased rooting in hard to root rootstocks. The favourable effects were highest in MM-109 where the number of rooted stools was increased from 25 to 80%. The increase in percent rooted stools was also recorded in M-4 and M-9 rootstocks. The NAA application was also found effective in crab apple, which is a hard to root dwarfing indigenous rootstocks but the effect was noticed only at 10,000 ppm NAA. The increase in the rooting in difficult to root rootstock species was due to synergetic effect of auxins in root initiation and growth. The differential behaviour of NAA may be attributed to the difference in levels of plant hormones in these rootstocks. The negative effect of higher concentrations of NAA may be due to antagonist effect. The application of NAA decreased rooting in easy to root rootstocks, i.e. MM-106 and MM-115. The detailed information regarding is mentioned in the following three tables.

Treatment	Percent rooting					
	MM-106	**MM-109**	**MM-115**	**M-4**	**M-9**	**Crab Apple**
Control	75.0	25.0	100.0	16.7	0.0	0.0
NAA(5,000ppm)	62.0	80.0	50.0	28.5	25.0	0.0
NAA(10,000ppm)	61.7	80.0	25.0	28.5	33.3	50.1

CD at 5%; Rootstock = 7.2; NAA=6.9; Int.= 7-4

Treatment	Number of roots/stool					
	MM-106	**MM-109**	**MM-115**	**M-4**	**M-9**	**Crab Apple**
Control	8.3	14.0	15.5	12.0	0.0	0.0
NAA(5,000ppm)	9.7	15.0	25.5	25	24.0	0.0
NAA(10,000ppm)	7.9	11.2	20.0	24.0	18.0	3.7

CD at 5%; Rootstock = 3.7; NAA=3.3; Int.= 3.9

Treatment	Average length(cm)					
	MM-106	**MM-109**	**MM-115**	**M-4**	**M-9**	**Crab Apple**
Control	14.4	5.8	19.6	10.8	0.0	0.0
NAA(5,000ppm)	13.7	11.6	10.3	11.2	14.6	0.0
NAA(10,000ppm)	13.8	6.7	8.4	9.0	7.6	15.0

CD at 5%; Rootstock = 4.1; NAA=3.1; Int.= 4.3

(c) Tissue Culture: Because woody species are generally difficult to root beyond the juvenile (seedling) stage by conventional methods, recourse to tissue culture methods offers an efficient *in vitro* regeneration method for fast multiplication of this important trees and an attractive alternative for species in which conventional propagation is impractical. Owing to high multiplication rates and potential for scale-up via bioreactor many researchers emphasized somatic embryogenesis. Cytokinin is important for callus formation and the ratio of cytokinin to auxin determines fate of callus if cytokinin: auxin ratio is high it promotes shoot proliferation while as low cytokinin: auxin ratio enhances root formation (Jain, 2013) [7]. In grapes & banana, BA and NAA are essential for establishment of explants. Gibberellic acid and salicylic acid are important for proliferation of stem explants (Fry and Street, 1980) [4].

Kumar *et al.,* 2016 [8] conducted an experiment to develop a protocol for rapid plant regeneration of apple. The leaf explants of apple cultivar *viz.* Golden Delicious were cultured for plant regeneration. The explants were cultured on MS media supplemented with different concentrations and combinations of BA with IBA and NAA as well as Kinetin with NAA. Maximum induction (80%) of leaf explants of Golden Delicious was observed in medium containing BA + NAA (1.0 + 0.5 mg/L). MS medium supplemented with different concentrations and combinations of BA, NAA and KIN were also employed for shoot regeneration. MS medium comprises BA (2.0 mg/L) and NAA (0.02 mg/L) in a particular concentration was best for maximum shoot regeneration from leaf calli, whereas, MS medium containing BA (2.0 mg/L) + NAA (0.02 mg/L) + GA_3 (0.4 mg/L) was best for maximum shoot elongation. The micro shoots were successfully rooted on half strength MS medium supplemented with IBA (1.0 mg/L) alone and 96.66% rooted plantlets were obtained using the medium. The *in vitro* plantlets were acclimatized in jiffy pots after rooting and established in the soil. The protocol of *in vitro* propagation, presented here is suitable and efficient for cost effective as well as timely production of plantlets for apple.

(2) Use of PGRs in nursery plant architecture management

Growth regulators help in manipulation of shape, size of crops without upsetting fruit quality and yield. Growth retardants are diverse group of chemicals which have common physiological effect of reducing stem growth by inhibiting cell division of sub apical meristem. SADH (paclobutrazol) is effective in reducing the growth of pear, peach, lemon, apple, litchi, apricot, plum and mango. Ethrel treatment is beneficial, in mango, grapes and avocado, for growth control. BA enhances sylleptic shoot formation over proleptic shoot formation. Split applications of BA and GA_{4+7} induced more and longer axillary shoots in apples. BA has significant influence on the crotch angle of branches of the apple trees. Growth regulators develop a higher number of branches, higher leaf area and better ratio between tiny roots and skeletal ones (Vahid, *et al.* 2016) [12]. Among the many growth regulators products tested over the past half century, products containing cytokinins (e.g., 6-benzyladenine) with or without gibberellic acid isomers (usually a mixture of GA_{4+7}) have proven the most useful for producing good quality feathered nursery trees. Branch induction in sweet cherry can be done by making a cut in above of bud then apply Promalin (BA +GA_{4+7}) in it along with paint or lanolin. The bud will develop into a bearing branch.

A new growth regulator, cyclanilide (CYC), has been tested for growth-related effects on nursery apple (*Malus* × *domestica* Borkh.). CYC treatment of difficult-to-branch cultivars such as ‘Cameo’ apple trees in the nursery increased the formation of well-developed feathers up to 6-fold and with minor or no effect on caliper or final tree height. CYC appears promising for lateral branch induction in nursery fruit trees. CYC displays significant additional advantages for nursery tree production. First, it does not produce significant long-term reduction of or deformation of growth of the terminal meristem even though it temporarily interrupts apical dominance.

Effect of cyclanilide (CYC) on lateral branching of Cameo/M7 apple nursery trees (Elfiving and Visser, 2006)

Treatment (mgL^{-1})	Feathers per cm^2 TCSA	Caliper (cm)	Tree height (cm)
Control	0.8d	1.8a	180a
CYC 100, applied once	4.6b	1.8a	166b
CYC 100, applied twice (1 week apart)	5.0ab	1.9a	177b
PR 500, applied once	1.5d	1.9a	167b
PR 500, applied twice (1 week apart)	4.4b	1.8a	1666
CYC 100, PR 500 (1 week apart)	5.5a	1.8a	164b
PR 500, CYC 100 (1 week apart)	2.5c	1.9a	172ab

PR= Promalin

TCSA= Tree cross section area.

Ramirez *et al.* (2010) [9] conducted the research to understand the effects of prohexadione-Ca (P-Ca) on the gibberellin changes at the apex and on the antioxidants and enzyme behaviour on ripening fruits of apple (*Malus* x *domestica* Borkh.). P-Ca was sprayed twice in the spring on ‘Red Delicious’/MM.106 apple trees when shoot growth reached 5 cm in length. The concentration dosages of the growth retardant were 0 (control), 125, 175 and 200 mg·L-1. The GCMS analysis of experimental samples showed the presence of gibberellins A_9 and A_{20} in P-Ca treated shoot tips; whereas gibberellins A_1, A_4, and A_7 were identified in control samples. The total antioxidant content in ripening fruits was increased with any concentration of P-Ca. A similar pattern was also observed in those fruits when vitamin C, catalase and peroxidase enzymes content were measured. Prohexadione-Ca applied to apple trees inhibits synthesis of GA_1, GA_4 and GA_7 in shoot apex; increases total antioxidant capacity, content of vitamin C and catalase and peroxidase activity in ripe fruit. It is possible that the increase of these enzymes activity may contribute to the process of vitamin C synthesis. P-Ca has been classified as an inhibitor of the synthesis of gibberellins GA_1, GA_4 and GA_7 which are biologically active.

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