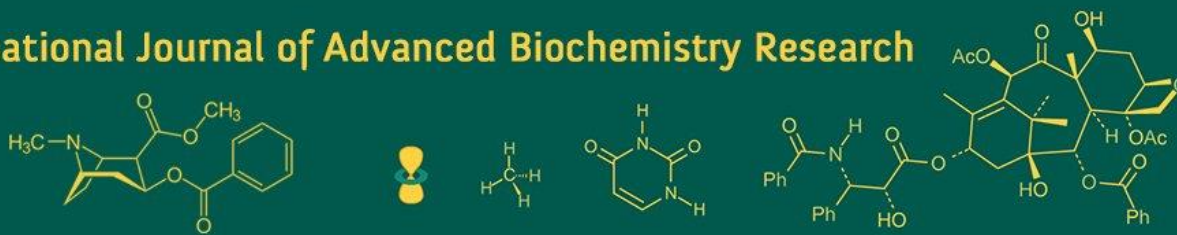


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Benefits of using plant growth regulators to enhance fruit production

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Abstract

Phytohormones are organic compounds naturally produced by plants that regulate growth and other functions at distant locations from their origin, even in minimal quantities. Examples of these plant growth regulators include *auxins*, *gibberellins*, *cytokinins*, and *ethylene*. Often, fruit production results in fruits of suboptimal quality. Therefore, applying plant growth regulators to fruit crops can significantly improve both yield and quality. For many cultivated plants, especially fruit-bearing ones, the use of growth regulators has become essential in agricultural practices. They are now applied externally to mitigate excessive fruit drop. Auxins and gibberellins, in particular, are commonly used to decrease fruit drop and enhance fruit quality. Various agents influence the transition of fruits from set to ripening and eventual consumer readiness. This review focuses on how plant growth regulators contribute to improving fruit development and production.

Keywords: Phytohormone, auxin, cytokinin, fruit development

Introduction

Plant growth regulators, or phytohormones, are naturally occurring chemical compounds in higher plants that regulate various physiological activities and growth processes, often at sites distant from their point of origin and in minute quantities. Examples include auxins, gibberellins, cytokinins, ethylene, and growth inhibitors [Pal *et al.*, 2019] ^[12]. Over the past 50 years, extensive research has been conducted to enhance fruit yield and quality through various methods such as plant varieties, propagation techniques, water management, training, and harvesting. Despite these advancements, subpar fruit quality remains a common issue. Therefore, applying plant growth regulators to fruit crops is a promising strategy for improving both yield and quality. These regulators have become an essential aspect of contemporary crop management practices. When used in appropriate concentrations, these organic compounds can significantly influence physiological processes in plants [Figure 1]. They are rapidly absorbed and distributed through plant tissues, affecting growth and development. The use of plant growth regulators has led to notable improvements in fruit crops, including enhanced branching, increased flower bud development, fruit thinning through abscission, reduced pre-harvest drop, improved fruit shape, and better regulation of vegetative growth. These improvements are particularly valuable for high-value fruit trees, where even minor adjustments in production practices can enhance quality and appeal [Kumari *et al.*, 2018] ^[10].

Plant growth regulators represent an innovative aspect of biotechnology, offering a new approach to managing plant biological functions to boost growth, yield, quality, and nutritional value while reducing biotic and abiotic stress. For instance, jasmonic acid, methyl jasmonate (MJ), and other jasmonates (JA's) play key roles in mediating plant responses to environmental stressors such as injury, insect, and disease attacks (Wasternack, 2007) ^[14]. Although these bio-regulators are widely used to improve various aspects of plant growth and production, the underlying molecular mechanisms remain partially understood.

Several classes of plant growth regulators include Auxins (IAA, NAA, IBA, 2,4-D, 4-CPA), Gibberellins (GA3), Cytokinins (Kinetin, Zeatin), Ethylene (Ethereal), Abscisic Acid (Dormins, Phaseic Acid), Phenolic Substances (Coumarin), Flowering Hormones (Florigin, Anthesin, Vernalin), and Growth Inhibitors (AMO-1618, Phosphon-D, Cycosel, B-999).

Types and Functions

Auxins are a class of plant hormones that include synthetic variants such as Naphthalene Acetic Acid (NAA), which are commonly used to aid vegetative propagation from stems and cuttings. The effectiveness of NAA in promoting plant growth depends significantly on its concentration and the timing of its application. NAA has been shown to enhance cellulose fiber production in plants [Suman *et al.*, 2017] [13]. Its application in various concentrations to fruit crops can effectively prevent fruit drop, particularly when applied after the blossoms have been fertilized.

In vitro propagation studies of bananas have demonstrated that Auxin I, which includes Indole Butyric Acid (IBA) and NAA, can improve root induction rates. A combination of IBA and NAA (1:1 ratio) led to better callus formation, root initiation, and partial success in banana *in vitro* propagation [Inoti, 2016] [7]. Bananas grown on Murashige and Skoog media with 2 M NAA developed roots more quickly and with more adventitious roots compared to those grown on media without NAA. However, roots grown without NAA showed increased transverse branching. This suggests the crucial role of auxins in banana maturation and the benefits of exogenous NAA application.

Auxin, particularly Indole-3-acetic Acid (IAA), is also known to delay the initial ripening of fruit, leading to a slower maturation process. The GH3 gene, which encodes IAA synthase, is involved in converting IAA to amino acids and interacts positively with Abscisic Acid (ABA) and sucrose to produce ethylene, thereby influencing the ripening process. For instance, treating grape berries with Benzothiazole-2-oxyacetic Acid (BTOA), an auxin-like compound, for two weeks, resulted in an increased ABA level, which affected the ripening process [He *et al.*, 2020] [6].

Gibberellins

Gibberellins play a crucial role in regulating various stages of fruit development, influencing the process from pre-pollination through to senescence [Cronjé RJ *et al.*, 2019] [4]. As fruits develop, their active metabolism and role as nutritional sinks make them highly responsive to hormonal regulation. After fertilization of the ovule, gibberellins contribute to fruit growth by affecting cell division and expansion.

For apple (*Malus domestica* B.) seedlings, breaking dormancy is commonly achieved through stratification in damp sand at 4-7 °C for three months. Combining this with gibberellic acid (GA3) treatment enhances results and reduces the chilling requirement. Exposure of apple seeds to GA3 (500 ppm) for 40 hours has been shown to improve germination rates, seedling growth, and sapling survival. Additionally, GA is highly effective in promoting seed germination when used in combination with other growth hormones. A mixture of GA3, salicylic acid (SA), jasmonic

acid, and 6-benzylaminopurine (BAP) during seed stratification not only promotes germination and seedling growth but also boosts chlorophyll content and improves PSII efficiency.

The role of gibberellins in citrus flowering and segmentation remains debated [Fahad *et al.*, 2015] [5]. While environmental factors are believed to primarily drive flower induction, some studies suggest that GA3 and the GA4+7 mixture can inhibit flowering rather than induce it. GA3's inhibitory effects are most pronounced in early autumn and late summer, with the greatest inhibition observed in January. Research indicates that applying GA before flowering can prevent the induction of citrus flowers.

Recent advances in research have led to the identification of key genes involved in gibberellin responses, paving the way for more targeted breeding programs. However, the impact of altering GA content or signaling pathways on various traits requires careful consideration. Differences between species, such as in the development of adventitious roots or flowering, highlight the need for species-specific approaches. GA's role in integrating cues like light quality or plant age into its responses, and its interaction with other phytohormones, underscores the complexity of its effects on fruit production [Castro-Camba R *et al.*, 2022] [3].

Cytokinins

Cytokinins are essential plant hormones involved in amino acid transport, cell growth, differentiation, and senescence. Their application can significantly impact fruit production. For instance, treating pears with 10 ppm of CPPU and 30 ppm of BA has been shown to increase fruit size. These treatments notably improved both the weight and size of pear varieties such as Spadona and Coscia. Additionally, research has demonstrated that applying BA at concentrations of 11 μM followed by 4.4 μM promotes optimal shoot multiplication in pear explants [Aremu AO *et al.*, 2020] [11].

Furthermore, a 10 ppm BA treatment enhances chlorophyll content in pear leaves, thereby delaying leaf senescence. BA also positively influences shoot growth in *Prunus* species and affects rootstock GF-677. Media containing TDZ or BAP have been found to increase the number of adventitious buds and buds per explant [Nowak *et al.*, 2002] [11]. In mangoes, 10 ppm of CPPU application results in improved fruit retention, increased fruit count per cluster, greater fruit weight, and larger leaf area. High levels of kinetin contribute to enhanced plant growth, characterized by larger stem diameters, more branches, and a greater number of leaves, while also reducing the risk of vegetative deformities. On the other hand, BA application can lead to increased fruit drop in pomegranate variety *Mirdula*. However, foliar application of 5 ppm CPPU in April has been found to boost fruit size and juice quantity in pomegranates.

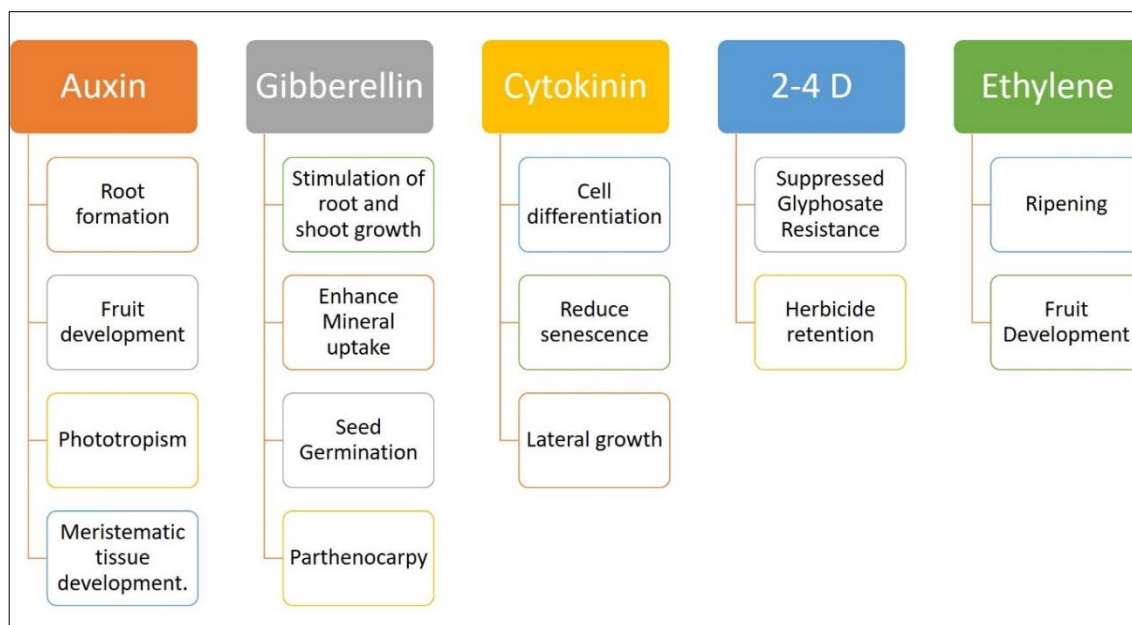


Fig 1: Role of Plant Growth Hormones on Fruit Production

2,4-D

The absorption of vitamins and minerals by developing plant organs and the longevity of buds are influenced by endogenous hormones and their balance. Exogenous application of 2,4-D or NAA has shown that abscission depends on the plant's endogenous auxin levels, with auxin transport continuing without apparent influence from ethylene.

In pummelo trees, 2,4-D and naphthalene acetic acid (NAA) have been effective in reducing fruit drop. An experiment involving five pummelo trees of similar age and size applied 2,4-D (20 and 40 mg L⁻¹), NAA (20 and 40 mg L⁻¹), or a control to ten mature branches per tree. Each treatment was applied twice: once at full bloom and again two months after fruit set. Results indicated that 20 mg L⁻¹ NAA (14.84%) and 40 mg L⁻¹ NAA (12.26%) significantly increased fruit retention after six months. Additionally, 2,4-D treatments at 20 mg L⁻¹ and 40 mg L⁻¹ resulted in higher levels of total nonstructural carbohydrates compared to the control (78.44 mg g⁻¹). Regarding fruit quality, 2,4-D at 40 mg L⁻¹ and 20 mg L⁻¹ produced the highest peel weights (435.55 g and 358.57 g, respectively), greatest peel thickness (20.25 mm), and the highest total soluble solids, compared to other treatments. Consequently, 20 mg L⁻¹ NAA applied twice significantly improved fruit retention.

For 'Hamlin' oranges, 10 ppm of 2,4-D effectively controlled pre-harvest fruit drop [Aziz *et al.*, 2020]. In research involving growth retardants such as CCC, daminozide, and paclobutrazol, the application of 500 ppm CCC was found to advance anthesis and fruit ripening by approximately 10 days. In another study on 'Sardar' guava, plant growth regulators like NAA, GA3, and CPPU were applied to 'Arumani' mango trees 14 days after blooming.

The application of CPPU (1-(2-chloro-4-pyridyl)-3-phenylurea) at 10 ppm yielded the best results in enhancing fruit retention, fruit count per cluster, fruit weight, volume, and leaf area.

Ethylene

Ethylene, a naturally occurring plant hormone, significantly influences the emergence, growth, and shelf life of various fruit crops. Both endogenous production and external application of ethylene contribute to its biological effects, which can occur at biologically active levels in harvested fruits either intentionally or inadvertently. The production and sensitivity to ethylene are heightened by various biotic and abiotic stressors as well as specific developmental stages of the plant [Khan *et al.*, 2020] ^[9].

Ethylene plays a key role in the ripening process of climacteric fruits, often driving a surge in its production that coordinates ripening phenomena. Generally, ethylene (C₂H₄) accelerates fruit ripening, enhancing flavor and taste. For example, tomatoes ripened off the plant with ethylene did not reach the same quality as those ripened on the plant, although the most significant fragrance molecule, (Z)-3-hexenal, increased by 31% and 17% with ethylene treatment.

While ethylene is beneficial for softening fruit tissues during ripening, it can also have negative effects on texture. It may cause undesired softening in cucumbers and peppers, and excessive toughening in asparagus and sweet potatoes. Generally, exposure to ethylene results in a loss of firmness in many ripening fruits and vegetables. Ethylene application is typically advantageous for fruits like apricots, avocados, melons, pears, and tomatoes. However, prolonged exposure can lead to excessive ripening and premature senescence.

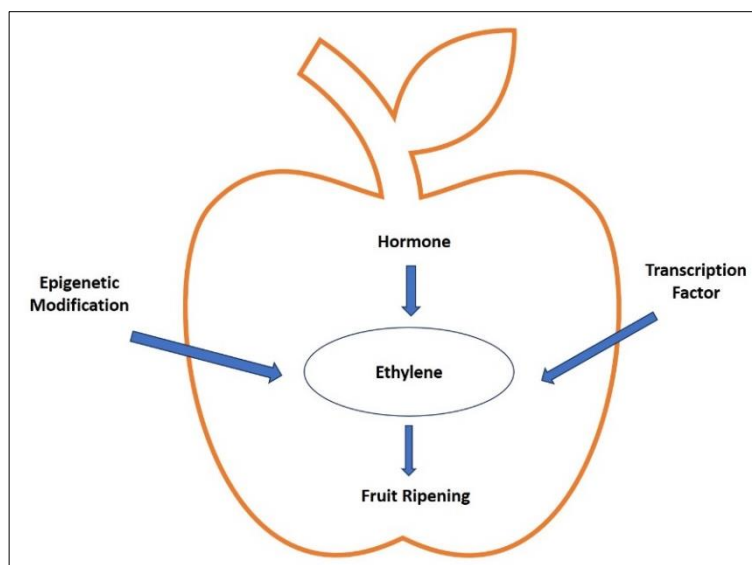


Fig 2: Role of Ethylene in Fruit ripening

Ethylene

In tropical fruits, once ripening begins, the concentration of inorganic ethylene (C_2H_4) quickly reaches a saturation point, and additional exogenous ethylene has limited effects on further promoting ripening. Due to the high resistance of both the flesh and skin of large fruits, such as apples (*Malus domestica*), bananas (*Musa* spp.), melons, and tomatoes, reducing the external ethylene concentration around these fruits has minimal impact on lowering the internal ethylene levels [Khadivi 2019]^[8]. The production rate of ethylene in these fruits significantly exceeds the rate of its diffusion, meaning that even if the external ethylene concentration is reduced to zero, the internal levels can remain higher than $100 \mu L L^{-1}$.

Therefore, reducing the external ethylene concentration through ventilation or ethylene scrubbers generally does not affect the ripening of fruit that has already progressed a few days into its climacteric phase. However, during the early stages of ripening, when internal ethylene levels are still low, enhancing the rate of ethylene diffusion using low-pressure storage or preventing ethylene production or action can effectively slow down the ripening process.

Conclusion

Auxins, such as Naphthalene Acetic Acid (NAA), are essential for promoting root formation and preventing fruit drop after fertilization. They enhance cellulose fiber production and can delay fruit ripening, which aids in managing harvest timings and extending shelf life. Gibberellins, including gibberellic acid (GA3), play a crucial role in various stages of fruit development, improving germination rates, seedling growth, and reducing chilling requirements. They also interact with other hormones to influence fruit quality, though their effects on citrus flowering can be complex. Cytokinins, like CPPU and BA, enhance fruit size and weight, and improve shoot growth and chlorophyll content. While they generally benefit fruit production, their application needs to be carefully managed to avoid issues such as increased fruit drop. Overall, the strategic use of these plant growth regulators can significantly enhance fruit production by optimizing growth and development processes.

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