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The potential of plant growth regulator (auxin) in enhancing fruit crop propagation via Stem Cuttings

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Abstract

Vegetative propagation via stem cuttings is a widely used technique in horticulture, particularly for clonal multiplication of fruit crops. The utilization of auxins such as naphthalene acetic acid (NAA) and indole-3-butyric acid (IBA) as plant growth regulators has shown great promise in improving the rooting capacity and general quality of stem cuttings. Auxins are essential for initiating and fostering adventitious root production, elongating and accelerating cell division, and controlling the patterning of the root meristem. Numerous studies across various fruit crops, including pomegranate, mulberry, fig, and dragon fruit, have reported improved rooting percentages, increased root quality parameters (root number, length, biomass), and enhanced shoot development when auxins are applied exogenously to stem cuttings. The physiological mechanisms behind auxin-induced rooting involve auxin biosynthesis, polar auxin transport, and downstream signaling cascades involving auxin-responsive genes and regulatory pathways. Optimizing auxin concentrations and application methods is critical for maximizing rooting success, as species-specific responses have been observed. The integration of auxin treatments with other propagation techniques can further enhance the efficiency and effectiveness of clonal propagation methods for fruit crops. Ultimately, the strategic use of auxins represents a promising and economically viable approach to improving the propagation of fruit crops through stem cuttings, contributing to sustainable and efficient horticultural practices.

Keywords: Plant growth regulator, auxin, stem cutting, rooting

Introduction

The reproduction, regeneration, and multiplication of plants is known as plant propagation. It enables plants to generate seeds, which facilitates progeny reproduction. It is a useful method of obtaining from trees specific features that may be lost through sexual propagation [1]. Although most plants are capable of sexual reproduction, some also reproduce vegetatively and by the use of hormones. It has the capacity to produce adventitious roots, which grow from other plant elements like the stem and leaves, enabling the creation of new plants from portions of existing ones. It is simpler to propagate young plants vegetatively. There are two ways to multiply plants: sexually and asexually. Fruit growing does not favor sexual (seed) reproduction because most fruit species are foreign pollinated, and some have a high dichogamy rate. Asexual reproduction involves the stacking, severing, cutting, and grafting of apomictic embryos [2]. Numerous fruit species and rootstocks are reproduced through the commercially significant process of vegetative generation in seedling production. The technique known as "vegetative production" allows for the creation of clones of plants by utilizing their organs, including their roots, branches, and leaves [3].

Cutting refers to the process of preparing plant parts by chopping off the roots, stems, branches, and leaves in order to create a new plant. Cuttings are used to create new plants by allowing them to develop roots and shoots in the right medium. The majority of fruit varieties, including quince, tea, olive, berry, pomegranate, vine and fig, can be readily multiplied through cuttings [4]. Furthermore, American grapevine rootstocks and clonal rootstocks used in fruit cultivation, as well as the cutting-based propagation method, are commonly employed in the propagation of ornamental plants. In horticulture, a plant cutting is a section of a plant used for vegetative (asexual) propagation (Goldschmidt & Joseph, 2023). A portion of the source plant's stem or root is inserted into a suitable medium, like damp soil. The process known as striking occurs when the right circumstances are met, causing the plant fragment to start growing as a separate plant from its parent.

New roots are produced by a stem cutting, and new stems are produced by a root cutting. Certain plants can be developed from leaf cuttings, which are fragments of leaf that grow into both stems and roots [6].

Cuttings are another term for the scions used in grafting. It's easier for certain plants than others to establish roots. Depending on the wood's age, multiple procedures are used for stem cuttings from woody plants. Softwood cuttings are utilized for immature leaves on fast growing stems. Such cuttings develop roots rather easily in many species. Stems with fully developed leaves and completed elongation growth are suitable for semi-hardwood cuttings. For fully grown stems, hardwood cuttings are utilized, and they are frequently propagated during dormancy [7].

Known by another name, "plant growth regulators," phytohormones are organic substances that are found in higher plants naturally and have trace amounts of activity. They control physiological processes, such as growth, at a site other than the plant's centre of synthesis. Thimmann coined the term "phytohormone" since plants produce these hormones. Auxins, gibberellins, cytokinins, ethylene, growth retardants, and growth inhibitors are examples of plant growth regulators (Hajam *et al.*, 2017). The hormones found in plants that were initially identified were auxins; gibberellins and cytokinins were later identified as well. In order to improve the quantity and quality of guava fruits, a great deal of research has been conducted in the nation over the past 50 years on a variety of topics, including varieties, propagation, irrigation, training, and pruning. It is general knowledge that low-quality fruits are produced (Suman *et al.*, 2017).

Stem cuttings are a useful tool for vegetative propagation because they keep the qualities of the parent plant, allow for faster flower and fruit production in plants established from cuttings, multiply quickly, and produce seedlings in a short amount of time. Auxin is a hormone found in plants that has a favourable effect on cutting quality and root growth [10]. A

plant growth hormone belonging to the auxin family, indole-3-butyric acid (IBA) is frequently utilized to stimulate roots. The auxin family also includes naphthalene acetic acid (NAA) and indole acetic acid (IAA), which are frequently utilized for root starting [11].

This review aims to elucidate the potential of plant growth regulators in enhancing the propagation of fruit crops via stem cuttings. It will explore the physiological mechanisms by which PGRs influence rooting, analyze empirical studies on various fruit species, and discuss practical considerations for their application in commercial propagation practices. By synthesizing current research findings, this paper will provide valuable insights into optimizing stem cutting propagation, thereby contributing to sustainable and efficient fruit crop production.

Role of auxin

It's well known that auxin promotes rooting in cuttings. The auxin most commonly used in commercial rooting is IBA. IBA and NAA are still the auxins most frequently used today for rooting stem cuttings and microcuts derived from tissue culture. Because auxins influence the establishment of primary, secondary, and adventitious roots, among other things, they are essential for controlling a plant's growth and development. The naturally occurring auxin found in plants is referred to as indole acetic acid (IAA). When compared to natural indole acetic acid (IAA), synthetic indol-3-butyric acid (IBA), the most often used auxin, is the hormone that most effectively promotes the formation of adventitious roots. (12). IBA controls a number of activities, including as fruit growth, vascular tissue differentiation, apical dominance, induction of roots, stem and coleoptile elongation, induction of cell division, and tropic movements like bending towards light. Commercially available synthetic versions of auxin include naphthalene acetic acid (NAA) and indole-3-butyric acid (IBA).

Table 1: Effect of auxin on rooting of different fruit crop cuttings

Fruit crop	Work done	Conclusion	Reference
Mulberry (<i>Morus alba</i> L.)	Effect of auxins and rooting media on rooting in stem cutting of mulberry (<i>Morus nigra</i> L.)	According to the research findings, the success of survival and rooting in Mulberry (<i>Morus alba</i> L.) cuttings was significantly influenced by the concentration of IBA and the type of rooting medium used. For Mulberry hardwood cutting propagation, the ideal treatments to take into consideration are 2000 ppm concentration of IBA and vermicompost rooting media.	[13]
Autumn Olive (<i>Elaeagnus umbellata</i> Thunb.)	Effects of some auxins on propagation by hardwood cutting of Autumn Olive (<i>Elaeagnus umbellata</i> Thunb.)	The results showed that the highest rooting percentage occurred as 70% in NAA 5000 ppm and IBA 5000 ppm treatment.	[14]
Fig (<i>Ficus carica</i> L.)	Effect of IBA and NAA concentrations and types of media on rooting and survival of cuttings in fig (<i>Ficus carica</i> L.)	The study determined that the rooting properties of fig cuttings grown in polyhouse conditions were strongly impacted by the application of the plant growth regulator IBA at varying concentrations in different types of media. When rooted cuttings were planted in media (soil + sand) in a 1:1 ratio, the treatment G3M3, or IBA @ 2000 ppm, showed the best morphological growth metrics.	[15]
Pomegranate (<i>Punica granatum</i> L.)	Influence of Biofertilizer and Auxin on Growth and Rooting of Pomegranate (<i>Punica granatum</i> L.) Cuttings	The treatment IBA 1500 ppm + NAA 1500 ppm + Biomix (T6) and treatment IBA 1500 ppm + NAA 1500 ppm + PSB + PGPR (T10) had significantly higher values on different shoot and root parameters in both cultivars, according to the effect of different auxin concentrations in combination with biofertilizers. Therefore, it is possible to draw the conclusion that, in both cultivars, the combined effects of auxin and biofertilizer produced better results in terms of root and shoot characteristics.	[16]
Acid-Lime Cv. Kagzi	Effect of Growing Media and Plant Growth Regulators on Rooting of Different Types of Stem Cuttings in Acid-Lime Cv. Kagzi	According to the study, acid lime Cv. Kagzi lime responded better to hardwood cuttings, cocopeat, and 500 ppm IBA in terms of inducing greater shooting and rooting parameters. The study's findings can be utilized to create a procedure for producing high-quality acid lime planting material from cuttings.	[17]

Dragon fruit (<i>Hylocereus undatus</i>)	Effect of growth regulators on rooting and shooting of stem cuttings in dragon fruit [<i>Hylocereus undatus</i> (Haworth) Britton & rose]	Based on the current experiment's results, it can be said that, out of the 12 treatments, IBA 7000 ppm and IBA 6000 ppm produced the best outcomes in terms of rooting and shooting parameters. The current investigation's results support the recommendation that the fast and affordable vegetative propagation method of growing dragon fruit using stem cuttings is a dependable approach for commercial plant production.	[18]
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Effective auxin application enhancing physiological responses in plant cuttings

Application of exogenous auxins is crucial for improving the quality and efficiency of rooting in stem cuttings, while IBA and NAA aid in encouraging adventitious rooting in cuttings. Using rooting hormones improves the quantity and quality of adventitious roots, facilitates the beginning of adventitious roots, and raises the overall percentage of rooting. IBA's conversion to IAA in plant tissue is what gives it its rooting-promoting properties. Carnation cuttings of several cultivars showed a notable increase in rooting percentage and other rooting parameters under treatment with IBA.

Many developmental processes are regulated by auxin distribution and transport. Acropetal auxin transfer frequently occurs from developing aerial tissues towards the PR. Tissue patterning and further root differentiation are initiated [19]. examined how PGRs affected pomegranate cuttings. The findings showed that IBA 500 ppm + Borax 1% produced the maximum rooting, number of roots, and length of roots for both semi-hard and hard wood cuttings. The highest field survival rates were retained by cuttings treated with IBA 500 ppm + Borax 1%, IBA 300 ppm + Borax 2%, and IBA 5000 ppm. Hard wood cuttings respond more favorably to the hormonal treatment than semi-hard wood cuttings do. Guava softwood cuttings treated with 1000 ppm IBA generated the longest shoots and the most roots. When semi-hardwood and softwood cuttings were treated with 1000 ppm NAA, the results included maximal

root length and early blooming [20]. A research was conducted on the "effect of hormones on the rooting of guava (*Psidium guajava* L.) Cv. Allahabad cuttings". The results showed that softwood cuttings treated with 1000 ppm solution of paclobutrazol had higher maximum root weight (1.46 g), more branches (3.44), higher survival (57.22%), and maximum sprouting (71.22%). The largest number of roots (59.66) and the tallest shoot (8.24 cm) were found in soft wood cuttings treated with IBA at 1000 ppm. Following treatment with 1000 ppm NAA, early sprouting (17.68 days) and maximum root length (12.81 cm) were observed in semi-hardwood and softwood cuttings, respectively [21]. Ayesha and Thippesha (2018) conducted a study to examine the effects of growth regulators on stem cutting roots in dragon fruit. The results demonstrated that the average number of roots per cutting (12.70, 21.74, and 46.68), the percentage of cuttings rooted at 30 and 60 DAP (33.66 and 57.5%), the length of the longest root at 30, 60, and 90 DAP (4.57, 10.65, and 23.07 cm), and the average length of the roots per cutting (3.95, 6.32, and 12.41 cm) were all found to be early in IBA 7000 ppm (14.54). the study of the possibility of producing rooted seedlings from *Prunus avium* stem cuttings. The results show that the growth hormone (IBA) dosage and the sample date have a significant effect on these incisions. The best results (60 percent rooting) were obtained with the January cutting and 50 mg/l of IBA. Even without the AIB treatment, 10% of the cuttings were successfully rooted in January [22].

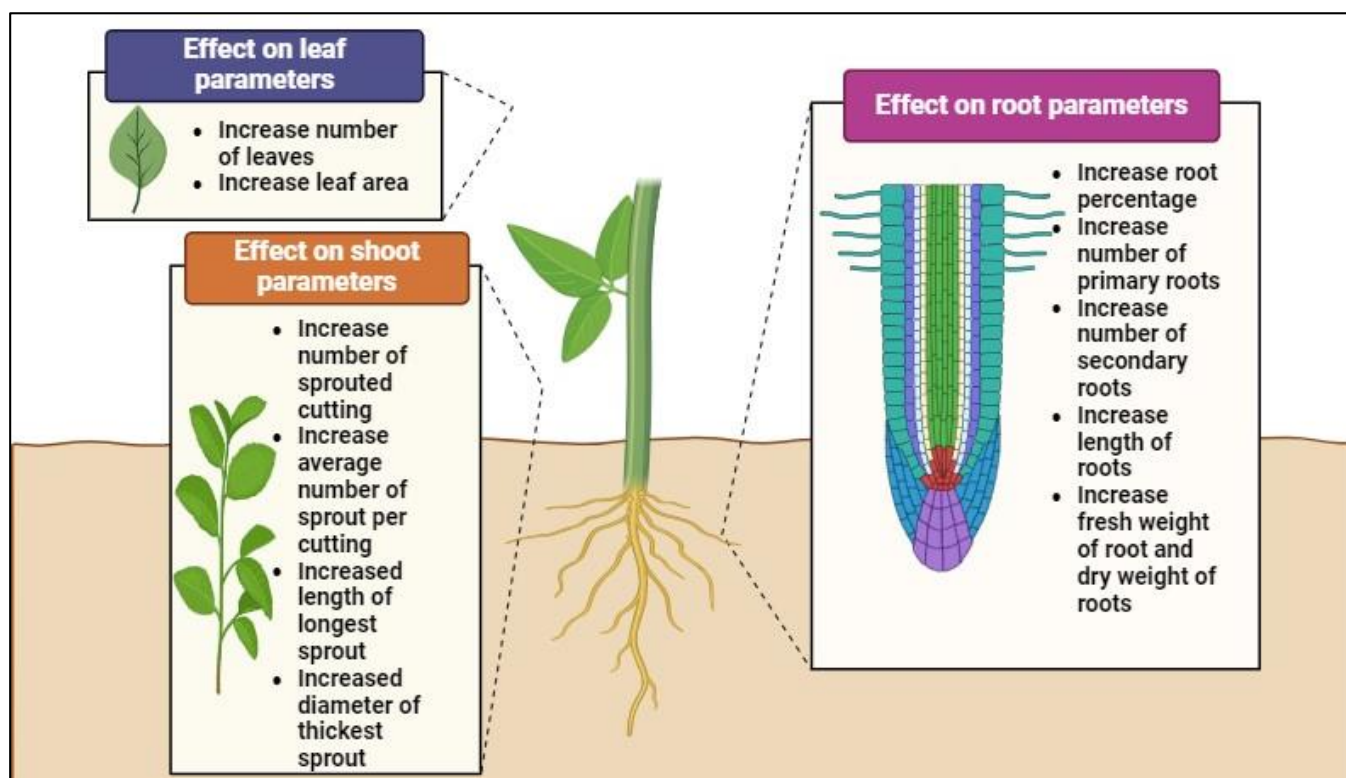


Fig 1: Effective auxin application enhancing physiological responses in plant cuttings

Auxin biosynthesis, transport and signaling

Auxins are obtained by a plant through auxin biosynthesis. Despite the complexity of the process involved in auxin production, our understanding of the several pathways and molecular mechanisms involved has significantly advanced in recent years. Auxin biosynthesis, represented by IAA, is shared by a number of plant species via a basic process that has been conserved throughout evolution. Researchers have looked into the consensus routes, genes, and metabolites involved in the production of IAA using biochemical, molecular, and bioinformatics techniques [23]. The two processes by which plants are thought to create IAA are Trp-dependent and Trp-independent, and both require the Trp precursor. Many routes, including as the indole-3-acetamide (IAM) pathway, the indole-3-pyruvic acid (IPA) pathway, the tryptamine (TAM) system, and the indole-3-acetaldoxime (IAOX) mechanism, have been proposed as being involved in Trp-dependent IAA formation [24]. In the Trp-dependent mechanism, indole-3-glycerol phosphate (IGP) is used in the chloroplast to convert chorismate to Trp. Trp is used as a precursor by tryptophan-2-monooxygenase, which is expressed by a gene related to the bacterial *aux1* gene, to produce IAM, which is then converted to IAA. Trp decarboxylase (TDC) is another cytosolic enzyme that converts Trp into the IAA precursor TAM. Through the IAOX pathway from Trp, the homologous cytochrome P450 enzymes CYP79B2 and CYP79B3 generate IAOX, an intermediate of IAA biosynthesis [25]. Young leaves and flowers are the principal sites of expression for these cytochrome P450 enzymes, which are predicted to be targeted to the chloroplast, as shown by the sites of auxin production. Overexpressing CYP79B2 resulted in increased levels of free auxin and distinct characteristics associated with auxin overproduction

in plants [26]. Conversely, twin mutants of *cyp79B2cyp79B3* had decreased IAA levels, which suggests their importance in auxin biosynthesis. Experiments involving labelling give support for the existence of a Trp-independent biosynthesis pathway. Further support for this came from the analysis of Trp biosynthesis mutants, such as *trp2-1* and *trp3-1*, which showed increased amounts of free IAA comparable to wild type (WT). Though the precise molecular mechanisms behind the Trp-independent pathway are yet unknown, it is believed that the primary building blocks for the synthesis of IAA are either indole or IGP [27].

Many developmental processes are regulated by auxin distribution and transport. Acropetal auxin transfer frequently occurs from developing aerial tissues towards the PR. This phase also promotes cell polarity and sets off further tissue patterning and root differentiation [28]. Two active pathways transport auxins to roots: the long-distance pathway transports auxins from the location of their synthesis, or from early aerial shoots to the base, through mature phloem. Cell-to-cell auxin transport is mediated by specific auxin influx and efflux carriers, but only over a certain distance [29].

In *Arabidopsis thaliana*, three main types of auxin transporters have been identified: the p-glycoproteins (PGP) ABC transporter family, pin formed (PIN) proteins, and like-*aux1* (LAX). PGPs are engaged in both auxin influx and efflux, whereas LAX and PIN proteins are responsible for auxin inflow and efflux, respectively. Observations indicate that auxins are moved via active auxin efflux from the cell and into the root [30].

Studies have indicated that mutations in *AUX1* impact auxin absorption and processes mediated by auxin, including as LR emergence, gravitropism, LR spacing, and RH formation [31].

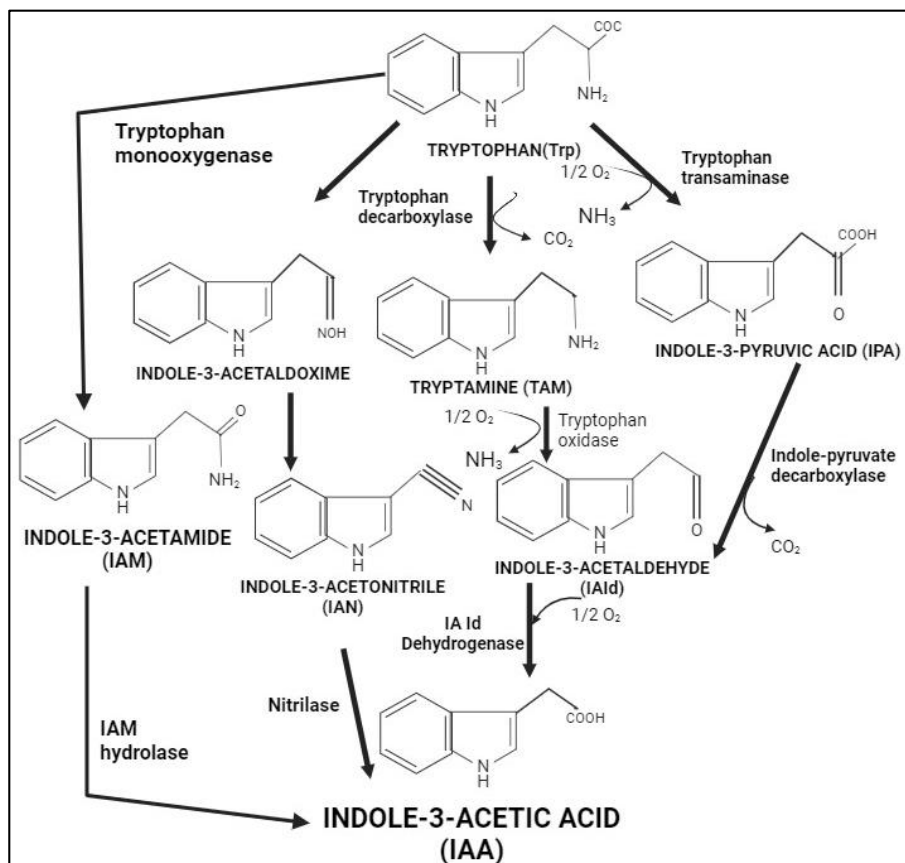


Fig 2: Pathway of tryptophan dependent synthesis of auxin (IAA) in plants

Auxin efflux carriers provide the auxin transport required to maintain auxin maxima in root development directionality. Examples of these carriers are PIN proteins on the plasma membrane with ten membrane-spanning domains. Experimental observations of single and multiple pin mutations altering gravitropic response, root meristem patterning, organogenesis, and vascular tissue differentiation suggest that auxin plays a critical role in these processes. Auxin transport inhibitors (ATI), such as 1-naphthylphthalamic acid (NPA), tri-iodobenzoic acid (TIBA), and 2-(1-pyrenoyl) benzoic acid (PBA), have also been shown to adversely affect auxin transport and distribution [32].

These ATIs block auxin efflux, which stops polar auxin from travelling across cells. Furthermore, it has been shown that a certain family of ATIs modifies the dynamics of actin in plants, offering a mechanism via which these drugs obstruct vesicle subcellular trafficking, including auxin efflux carriers. AUX1 and PIN are more engaged in auxin transport than PGP are. In addition to these transporters, research have indicated the involvement of the genes plethoral1 (PLT1) and plethoral2 (PLT2), which encode apella2 (AP2) class transcription factors involved in quiescent centre (QC) specification and stem cell activity that promotes root growth [33]. In order to affect auxin distribution and root growth, they also regulate the expression of a subset of PIN genes. Additionally, the location and retention of PIN proteins on the plasma membrane are essential and dynamic processes. It is

essential for maintaining cell polarity, differentiating auxin distribution, and tissue patterning. Although the precise nature of cell polarity remains unclear, several recent studies have provided more insights into the regulatory mechanism governing auxin transport [34].

Two pathways allow vesicle-cycling machinery to rapidly target PINs: GNOM, which encodes the ADP-ribosylation factor GTPase guanine exchange factor (ARF-GEF), and reversible protein phosphorylation involving pinoid kinase (PID) and protein phosphatase 2A (PP2A). PID/PP2A facilitates PIN apical-basal polar targeting, although basal targeting requires GNOM activity. Studies reveal that PID's antagonistic effect on PIN's phosphorylation and partial colocalization with PID and PP2A mediates its polar distribution [35].

Furthermore, it is suggested that polar PIN targeting, which is dependent on both PID and GNOM, is essential for a number of developmental processes, including root formation. Auxin must first enter the cell through the auxin import carrier AUX1, in order for it to be transported out. The auxins can then attach to the auxin receptor, transport inhibitor response 1 (TIR1) (Figure.3), which will then trigger the auxin signalling cascade. Numerous endogenous and external cues combine to initiate a complicated signalling system that drives the last stage of auxin-mediated root growth [36]. Over the past ten years, there has been a notable acceleration in the quest to understand the role of auxin signalling in root development due to the introduction of novel molecular and genetic techniques.

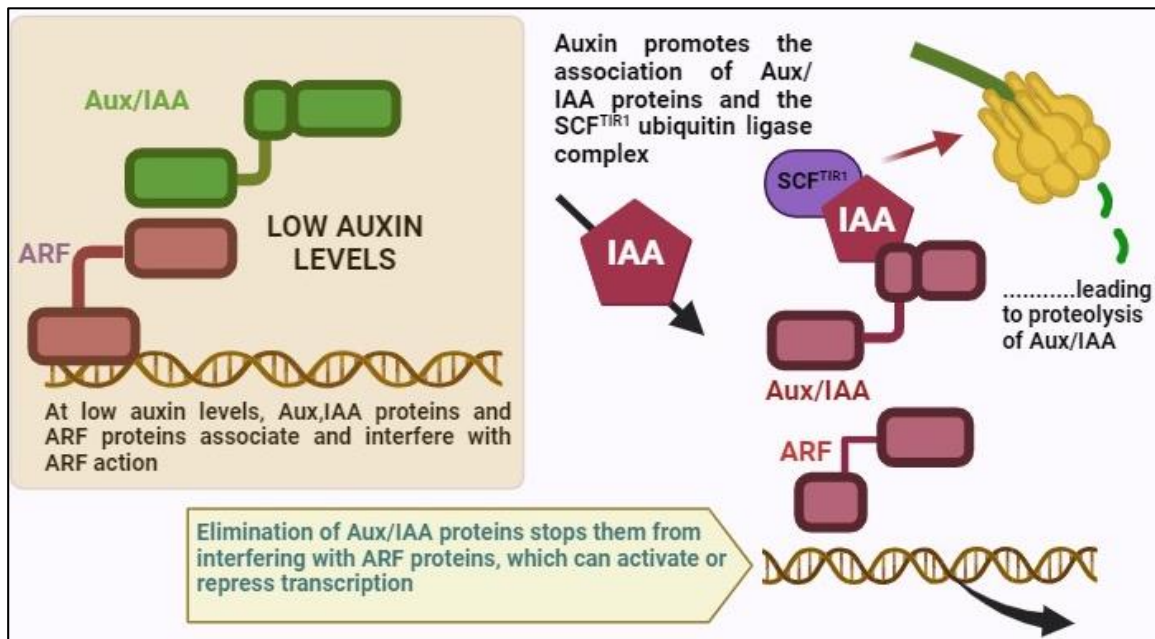


Fig 3: The auxin signalling pathway

Numerous endogenous and external cues combine to initiate a complicated signalling system that drives the last stage of auxin-mediated root growth [37]. Over the past ten years, there has been a notable acceleration in the quest to understand the role of auxin signalling in root development due to the introduction of novel molecular and genetic techniques. Auxin signalling is known to stimulate root growth and development through ubiquitin-dependent proteolysis [38].

Transcription repressors and auxin response factors (ARF), which can either activate or repress transcription depending

on the type of ARF involved, are members of the auxin/indole-3-acetic acid (AUX/IAA) protein families. ARF 1 and 2 are thought to serve as transcriptional repressors, but ARF 5, 7, 8, and 19 are known to function as transcriptional activators because they lack a Q-rich middle region. Consequently, ARF is triggered, and it subsequently binds to the promoter of auxin-responsive genes that are implicated in the growth and development of roots [39].

Numerous mitogen-activated protein kinases (MAPKs) have been demonstrated to play a role in the control of polar auxin transport and root development. MAPK kinase-7

(MKK7), a member of the oxidative stress MAPK family, has been linked to the architecture of the root system via the negative regulation of polar auxin transport. Furthermore, another MAPK known as MAPK 12 (MPK12) negatively regulates indole-3-butyric acid-responsive protein 5 (IBR5), which is predicted to encode a dual-specificity MAPK phosphatase [40]. It has been shown that IBR5 phosphatase dephosphorylates MPK12 in *A. thaliana*, rendering it inactive. The inhibition of MPK12 leads to the overexpression of auxin-responsive genes involved in root growth, revealing the involvement of MPK12 as novel negative regulator of auxin signaling. Furthermore, research has been done on the role that calcium (Ca^{2+}) plays in controlling adventitious root growth [41]. It has been shown that the availability of intracellular and external calcium pools affects the response of plants to IAA and nitric oxide (NO). Furthermore, it was demonstrated that IAA triggered a MAPK cascade through a cGMP-independent but NO-dependent mechanism. This investigation assessed the role of Ca^{2+} and the control of Ca^{2+} dependent protein kinase (CDPK) activity during IAA and NO induced adventitious root development in cucumber explants. The transitory expression of constitutively active MAPKKK (ANP1) induces MAPK activity, which results in a reduction in the transcription of the auxin-responsive gene. This suggests that MAPK is stimulated in order to cause the downregulation of auxin-responsive genes [36].

Conclusion

The application of auxins as plant growth regulators holds significant potential in enhancing the propagation of fruit crops via stem cuttings. Through extensive research and practical applications, it has been demonstrated that auxins play a crucial role in initiating and promoting root development, which is vital for the successful establishment of new plants. The use of synthetic auxins, such as Indole-3-butyric acid (IBA) and Naphthaleneacetic acid (NAA), has shown consistent and positive effects on rooting percentages, root quality, and overall plant vigor across a wide range of fruit species. The physiological mechanisms behind auxin-induced rooting involve the activation of root-promoting genes, enhancement of cell division and elongation, and the regulation of adventitious root formation. These processes collectively improve the efficiency and effectiveness of clonal propagation methods, making them more viable and reliable for commercial fruit production. The optimal concentrations and application methods, however, can vary among species, necessitating species-specific protocols for maximum efficacy. Moreover, the integration of auxin treatments with other propagation techniques, such as mist systems and substrate optimization, can further enhance rooting success and plant development. The economic benefits of using auxins are also notable, as they can significantly reduce the time and cost associated with plant propagation, leading to more rapid multiplication and availability of high-quality planting material. In conclusion, the strategic use of auxins represents a promising and practical approach to improving the propagation of fruit crops through stem cuttings. Future research should continue to explore the species-specific responses and refine application techniques to maximize the benefits of auxin treatments. Embracing these advancements can contribute to more sustainable and efficient horticultural

practices, ultimately supporting the global demand for fruit crops.

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