



Synthetic Auxin and Cytokinin like Plant Growth Regulators

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Abstract

Phytohormones are necessary for proper manifestation of organs in plant throughout the life cycle. Natural auxin and cytokinin are known to be endo-synthesized in apical meristem and leaf respectively and translocated in the plant portions also to the point site of action. But, the amount available is dependent on the environmental factors including light and temperature. However, synthetic auxin and cytokinin like compounds are now available and marketed appreciably to mitigate the need by external use to maintain the actual requirement and interaction. Beneficial effect of supplementing synthetic plant growth regulators had been gathered from researches are numerous.

Auxins and Cytokinin like natural and synthetic compounds are synthesized from mainly from Tryptophan type aromatic acids and Terpeneoids respectively. From Gene Regulatory Networks and others it reveals that auxins acts through AUX/IAA family and ABC1 family genes. Cytokinin/Kinetins manifested through IPT-SARK genes.

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Introduction

Hormones take part in plant development in all stages from seed germination, through vegetative growth, flower induction to maturity and decomposition. Auxin and cytokinin are some of those important hormones having a cardinal role within plants. Some of the functions of auxin are cell elongation, fruit growth stimulation and development but also apical dominance regulation (McDonald, 1997) ^[65]. The basic functions of cytokinin are stimulation of cell division in meristems, prevention of senescence and elimination of apical dominance (Sujatha and Reddy, 1998) ^[103]. A characteristic feature differentiating both hormones is that they regulate photosynthesis and physiology in different parts of a plant (Mikos-Bielak, 2005) ^[66]. A characteristic feature of plant hormones is their cooperation with other hormones. One of the examples can be exogenous auxin and cytokinin, in particular in larger concentrations, both stimulating the biosynthesis of ethylene in tissues (Lorteau *et al.*, 2001, Khan *et al.*, 2002) ^[62, 53]. It proves that a lot of processes which are attributed to the effect of those hormones can be in fact the reaction of the plant to a high concentration of ethylene. Another example can be a mutual influence of cytokinin and auxin, for example, in the stimulation of cambium activity and the vascular tissue formation. It results in a better supply of tissues in photosynthesis products and raises immunity to stress factors, like water stress (Aldesuquy, 2000) ^[6].

Plant Growth Regulators are natural hormones or synthetic analogues that influence various aspects of plant growth and development, such as cell division, expansion, and response to environmental stress. They are applied to plant parts to enhance resilience, improve growth under challenging conditions, and increase crop yield in agriculture, viticulture, and horticulture.

Enhancing crop yield and horticultural characteristics under optimal condition and while mitigating yield loss under stressful conditions is a major challenge in the exploration of artificially synthesized PGRs. Nonetheless, natural PGRs appear to sustain a harmonious equilibrium between augmenting stress resistance and promoting growth and development.

The synthesis of PGRs may serve as a viable solution to circumvent this issue. Further, these are valuable for investigating the molecular mechanism and optimize chemical structure, focus on synthesis novel PGRs that exhibit stronger bioactivity and simpler synthetic route.

It is indicated that the use of plant growth regulators, regulation of plant metabolism, control of flowering, effects on fruit set and development, control of abscission, Breaking of Seed dormancy and seed germination, enhancing and regulation of flowering, controlling flower drop, fruit thinning, fruit setting, parthenocarpy, post-harvest management and extending the shelf life of horticultural crops.

Auxins are plant growth regulators, similar to CKs, and are mainly involved in root/shoot formation and relative growth (Sachs, 2005) ^[90]. Studies have shown that auxins work together with CKs in various cellular or physiological processes such as cell cycle progression, cell expansion, apical dominance, leaf development, and embryonic development during seed maturation (Tomas *et al.*, 2009; Jurado *et al.*, 2010) ^[107, 50]. Indeed, auxins under environmental stresses influence the plant growth responses. In contrast, subsequent alterations in auxin homeostasis due to such environmental changes can result in distorted growth and development in plants, causing altered morphogenesis. Such stress-induced morphogenic responses are well known. However, to approach the maximal crop yield potential, it is also necessary to overcome the limitations on yield caused by the natural growth regulatory systems. The metabolic reactions in plants are controlled both by the supply and conversion of nutrients and by their endogenous (internally derived) hormonal pattern. The main groups of natural plant hormones are auxins, gibberellins, cytokinins, abscisins, and ethylene. An understanding of the mode of action of plant bioregulators on the molecular level requires the identification of the receptor site for each regulator, as well as the elucidation of the subsequent reactions.

Auxin and Cytokinin

Auxins are more often found in younger plant parts (juveniles and seedlings) and play an important role in the early stages of plant development (George *et al.*, 2008) ^[31]. Because light directly affects them, auxins are responsible for phototropism (growth of plant in response to light); however, auxins also effect apical dominance, lateral root initiation, angiogenesis and gravitropism (Davis, 2010) ^[22]. Auxins interact with salicylic acid and abscisic acid to regulate plant growth during abiotic stress (Park, 2007) ^[80]. It is believed that foliar application of natural or/and synthetic auxins improves physiological processes.

Auxins (AUX) act from the embryo formation until tropic stimulus processes, but are known as growth hormone due to their role in cell elongation. The response to auxin includes regulation of a broad number of genes. In plants the predominant auxin is indole acetic acid (IAA). There is a large biosynthesis of auxin in young tissues on the shoot as well in the root meristematic apices. Auxin is distinguished from the other hormones by its specific and active transport, which is one of the factors that influence the activity of this hormone (Spartz and Gray, 2008) ^[102].

Cytokinins are able to stimulate cell division and induce shoot bud formation in tissue culture. They usually act as antagonists to auxins. (Cytokinins are N6 substituted derivatives of the nitrogenous purine base adenine.)

Cytokinins most used in tissue culture include zeatin, adenine, 6-(g,g-dimethylallylamino) purine (2 iP) and kinetin. Cytokinins often inhibit embryogenesis and root induction.

Cytokinins are often used to stimulate growth and development, zeatin, kinetin, BAP, 2, P and pyranilbenzyladenine (PBA) being common. They usually promote cell division, especially if added together with an auxin. In high concentration (1 to 10 mg.ml⁻¹), they can induce adventitious shoot formation, but root formation is generally inhibited. They promote auxiliary shoot formation by decreasing apical dormancy (Slater *et al.*, 2005) ^[90].

The CKs are key plant hormones often considered ABA antagonists and auxin antagonists/ synergists in various processes in plants responsible for plant growth, development, and tolerance against different abiotic stresses (Pospíšilová, 2003; Danilova *et al.*, 2016) ^[82, 20]. Besides, other hormonal pathways (e.g., ABA), CKs are activated when a plant is exposed to salt stress. By interacting with other plant hormones like auxins and ABA, CKs can considerably increase salt stress tolerance (Iqbal *et al.*, 2006) ^[44]. Cytokinin (CYT) is a phytohormone that participates in events in the course of whole plant ontogeny, from fecundated ovule to senescence and death. It is present in processes such as cell division, shoot initiation and growth, senescence delay and photomorphogenic development, control of chloroplast division and growth, modulation of metabolism and morphogenesis in response to environmental stimulus (Hirose *et al.*, 2007) ^[42].

Auxin and Cytokinin like synthetic plant growth regulators

PGRs are useful because they can in some way modify plant development. This may occur by interfering with the biosynthesis, metabolism, or translocation of plant hormones, or PORs may replace or supplement plant hormones when their endogenous levels are below that needed to change the course of plant development.

Commonly used synthetic auxins include: 2,4-Dichlorophenoxyacetic acid, 2-methoxy-3,6-dichlorobenzoic acid (dicamba); indole-3-butyric acid; 2-methyl-4-chlorophenoxyacetic acid (MCPA), 1-naphthylacetic acid (NAA), 2-naphthylacetic acid (NOA), 4-amin-2,5,6-trichloropicolinic acid (picloram). The synthetic auxins include the following herbicide families: benzoic acids, phenoxycarboxylic acids, pyridine carboxylic acids, and quinoline carboxylic acids that act similar to that of endogenous plant auxin. Also Ivin (N-oxide-2,6-dimethylpyridine), Methyur (sodium salt of 6-methyl-2-mercapto-4-hydroxypyrimidine), Kamethur (potassium salt of 6-methyl-2-mercapto-4-hydroxypyrimidine) are known to act likewise.

Commonly used cytokinins include 6-benzylaminopurine (BAP), [N6-(2-isopentyl) adenine] (2iP (IPA)), 6furfurylaminopurine (Kinetin), 1-phenyl-3-(1,2,3-thiadiazol-5-yl) urea (Thidiazuron); 4-hydroxy-3-methyl-trans-2butenylaminopurine (Zeatin), as synthetic analogues, naturally occurring cytokinins and substituted phenylurea-type cytokinin.

Use of auxin and cytokinin like plant growth regulators

PGRs were encapsulated in liposomes using a thin film hydration method (De and Venkatesh, 2012) ^[23]. The following wall (phosphatidylcholine)-to core (PGRs) ratios

were used in the encapsulation: IAA: phospholipid (1:8) and GA-like substances: phospholipid (1:5). The phospholipids and PGRs were initially dissolved in 95% ethanol. The mixture was then dried in vacuum at 55 °C to form a thin lipid film. The film was rehydrated with phosphate buffer (0.5 M, pH 7.2) to produce multi-lamellar liposomal vesicles containing the PGRs. It was then homogenized and sonicated for 30 min at room temperature. Maltose, a cryoprotectant, was then incorporated prior to lyophilization. The freeze-dried residue was stored at 4 °C prior to use.

Hormones like cytokinins and auxins are chemicals that regulate plant growth. As such, they shape the plant and affect seed growth, time of flowering, sex of flowers and the senescence of leaves and fruits. Also, they affect the tissues that grow upward and downward, the formation of the leaf and the growth of the stem (Helgi opik and Stephen, 2005) [39]. Indole butyric acid (IBA) and naphthalene acetic acid (NAA) which are auxins compounds that positively influence root initiation and in conjunction with cytokinins, they control growth of stems, roots, flowers and fruits (Helgi opik and Stephen, 2005) [39]. Cytokinins which include 6-benzylamino purine (BAP) and zeatin are group of chemicals that influence cell division and shoot formation.

Synthetic growth regulators that cause development of fruits in plants include 4-Chlorophenoxyacetic acid and 2-naphthoxyacetic acid. These chemicals are most effective on fruits with many ovules, e.g., tomato, squash, eggplant and fig. However, these chemicals usually are ineffective in peach, cherry, plum, and other stone fruit. The auxin family serves a number of functions in plants, such as cell and stem elongation, leaf expansion, increased rooting and changes in fruit abscission. Some yield response has been noted when certain auxins have been applied to cotton, probably due to greater boll retention. Other reports have shown no yield response (Nickell, 1994) [75].

Auxins such as NAA have been used since a long time to improve fruit quantity and quality of many deciduous fruit trees. Antonio and Bettio (2003) [9] showed that application of NAA at the rate of 30 mg l⁻¹ led to increase in fruit size and delay in harvesting period of peach CV. Diamante. Ruth *et al.* (2006) [79] studied the effect of synthetic auxins on fruit size of five cultivars of Japanese plum and found that application of 30 mg l⁻¹ of NAA at the beginning of pit hardening caused an appreciable and significant increase in fruit size. Amiri *et al.* (2012) [7] showed that 400 mg l⁻¹ of NAA application reduced the preharvest fruit drop, and an increase in fruit size was noticed in Satsuma mandarin. Application of NAA @ 400 ppm during the last week of April and first week of May to achieve maximum guava yield was suggested (Abbas *et al.*, 2014) [1]. The effect of growth regulator NAA on fruit drop, yield and quality of mango cultivar Langra was noted with 200 ppm of the chemical (Haidry *et al.*, 1997) [37]. Application of NAA significantly increased trigonelline and mucilage content of seed in fenugreek (Danesh Talab *et al.*, 2014) [19]. In appropriate concentration NAA affects the growth and yield of a number of plants, viz. tomato (Chhonkar and Singh, 1959) [16], bitter gourd (Jahan and Fattah, 1991) [46] and cowpea (Ullah *et al.*, 2007) [109]. Planofix (NAA) had a significant effect on plant height, number of fruiting branches, volume of boll and yield of cotton (Abro *et al.*, 2004) [103]. NAA has been used for the enhancement of growth and yield of cereals (Lilani *et al.*, 1991) [60]. Application of 20 ppm of NAA showed better performance in enhancing the straw and grain yields of wheat cultivars

(Alam *et al.*, 2002) [5]. Growth and yield parameters of rice were significantly promoted in response to various auxin levels (Zahir *et al.*, 1998) [119]. Reports regarding the growth and yield aspects with NAA on cereal plants, including rice are available (Mishra and Sahu, 1957; Chaudhuri *et al.*, 1980; Muthu Kumar *et al.*, 2005) [67, 15, 70].

NAA is a high-efficiency auxin-like plant growth regulator. When applied in low concentrations as foliar spray on plants, it is transported basipetally downward slowly to initiate adventitious roots and better root activities (Zhi-Guo *et al.*, 2012) [121], thus enhancing nutrient uptake. It also improves cell elongation and cell division (Campanoni and Nick, 2005) [13] and thus growth is enhanced. In small concentrations, it delays senescence. Hence, crop growth is found to increase significantly. Keeping the above in consideration, it may be inferred that application of NAA has the following effects: (i) Efficient root activities which improve nutrient uptake for better growth of rice plant; (ii) Improves growth parameters effectively; (iii) Most of the yield attributing factors are enhanced; (iv) Significant increase in grain yield; (v) Delayed senescence to improve mobilization of assimilates from source to sink (Basuchaudhuri, 2016) [11].

It is also known that the organo-silicon growth regulator Energia-M (orthoethoxy acetic acid triethyl ammonium salt+chloromethylsilatrane) has a high immunoprotective activity, slows down premature ageing and death of plants from extreme environmental factors. Growth regulator Energia-M (auxin, silicon) increases catalase and peroxidase activity, which contributes to the intensity and productivity of the general metabolism in the plant and protection from damage by hydrogen peroxide formed as a result of redox reactions. The activity of polyphenol oxidase upon treatment with growth regulators decreased in all variants as compared with the control. Probably, the decrease in polyphenol oxidase activity is compensated by an increase in catalase and peroxidase, since antioxidant defense enzymes function in a complex under conditions of an increased content of highly active molecular products. When the antioxidant defense enzymes are activated, the level of malondialdehyde in the leaves decreases, which is probably associated with the activity of peroxidase and catalase. New generation synthetic plant growth regulators initiated the tuberization process, which influenced the increase in the yield of the *Solanum tuberosum* (Sawicka, 2008) [93].

Functions of NAA are stimulation of cell division, cell elongation, elongation of shoot, photosynthesis, RNA synthesis, membrane permeability and water uptake involved in many physiological processes like prevention of preharvest fruit drop, flower induction, fruit set, delayed senescence and prevention of bud sprouting, leaf chlorophyll content and increased yield in fruit crops. An exogenous application of naturally occurring or synthetic plant growth regulators affects endogenous hormonal pattern of the plant either by supplementation of sub-optimal levels or by interaction with their synthesis, translocation or inactivation of existing hormone levels.

Auxins stimulate cell elongation and influence a host of other developmental responses, such as root initiation, vascular differentiation, tropic responses, apical dominance and the development of auxiliary buds, flowers and fruits. Auxins are synthesized in the stem and root apices and transported through the plant axis. The principal auxin in plants is indole-3-acetic acid (IAA). Several other indole derivatives, all as precursors to IAA, are known to express

auxin activity, probably by converting to IAA in the tissue. Auxins in plant tissue culture are used to induce callus from explants, and cause root and shoot morphogenesis. Auxins are often most effective in eliciting their effects when combined with cytokinins (Soil and Plante Library, 2024) ^[101].

The effect of synthetic plant growth regulators: Ivin (N-oxide-2,6-dimethylpyridine), Methyur (sodium salt of 6-methyl-2-mercapto-4-hydroxypyrimidine), Kamethur (potassium salt of 6-methyl-2-mercapto-4-hydroxypyrimidine) on plant growth in field, laboratory and in vitro conditions, crop productivity and adaptation to stress factors was studied. The growth regulating activity of plant growth regulators Ivin, Methyur and Kamethur was compared with the growth regulating activity of the auxin IAA (1H-Indol-3-yl) acetic acid. It was found that the treatment of seeds before planting

in the soil with water solutions of Ivin, Methyur and Kamethur, used at a concentration of 10⁻⁷M, contributes to an increase in morphological parameters (length of shoot and root, fresh weight of plant) of sunflower (*Helianthus annuus* L.) cv. Bastion grown in field conditions for 3 months.

The obtained results suggested that the high growth regulating activity of Ivin, Methyur and Kamethur is explained by their specific auxin-like stimulating effect on the proliferation, elongation and differentiation of plant cells, which are the main processes of the formation and development of plant shoots and roots, as well on the biosynthesis, metabolism and signaling of endogenous auxins in plant cells (Fig.1) (Tsygankova *et al.*, 2023) ^[108].

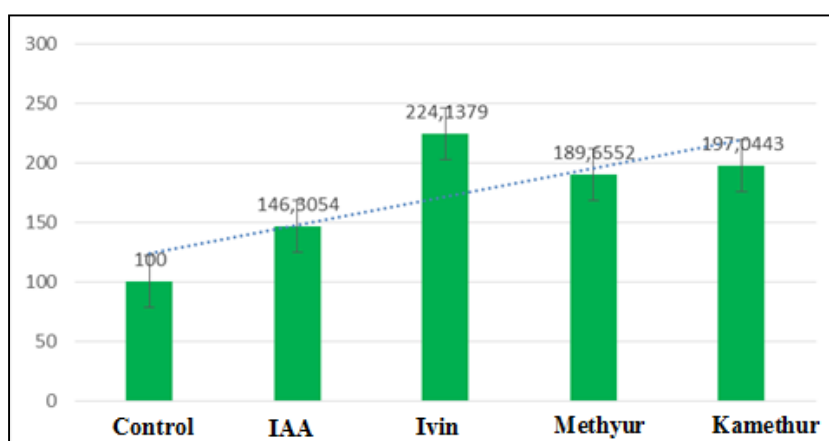


Fig 1: Morphological parameters of sunflower (*Helianthus annuus* L.) variety Bastion grown for 3 month in field condition (average fresh weight of plant (%)).average fresh weight of plant (%)

The effectiveness of Nano-IAA in the rooting response of coffee nodal cuttings var. Robusta was evaluated in collaboration with the National Coffee Research, Development, and Extension Center (NCRDEC) of Cavite State University in Indang, Cavite. Harvested shoots were cut into single-nodal cuttings 4–6 cm long. The cut was made such that the end portion or base is pointed while the top portion or apex is flat. The following treatments, with three replicates per treatment and 12 nodal cuttings per replicate, were used: water (negative control); Hormex® (commercially available synthetic hormone with 0.24% naphthalene acetic acid or NAA); Quickroot (commercially available synthetic hormone with indolebutyric acid or IBA); and Nano-IAA (1, 3, 5, and 10 ppm) (Fernando *et al.*, 2024) ^[29] are clearly effective.

Plant tissue culture, an aspect of agriculture biotechnology provide a method for the mass clonal propagation of plants via in vitro regeneration, it is also a tool for their germplasm conservation as well as for reforestation and tree improvement (Raddy *et al.*, 2001; Amoo and Ayisire, 2005) ^[84, 8]. However, germinated embryo is a model system which has application in higher plant tissue, organ culture and genetic transformation, since the regeneration of specific organs may be effectively manipulated through the use of germinated embryos in conjunction with specifically controlled in vitro condition and exogenously applied plant growth regulators. Many problems hindering the improvement of in vitro plant systems are potentially removed (Jaime and Teixeira, 2003) ^[48]. Also, plants which are obtained through organogenesis or somatic or zygotic

embryogenesis are frequently free of pathogens that might have systematically infected the mother plant (Thottapilly *et al.*, 1992) ^[106].

Auxins generally cause cell elongation and swelling of tissues. Use of 2,4-dichlorophenoxy acetic acid should be limited as much as possible since it can induce mutation. Although a wide variety of auxin such as 2,4-D, (2,4,5 trichlorophenoxy) acetic acid (2,4,5-T), NAA, IAA picloram and dicamba have been used in cereal tissue culture, 2,4-D at a concentration of 1 to 2.0 mg.L⁻¹ have been found to be most satisfactory for the production of calli capable of subsequent morphogenesis (Wang and Zapata, 1987) ^[114]. Heyser *et al.* (1983) ^[41], however, reported the use of high concentration of auxin (20 mg.L⁻¹; 2,4-D) for embryogenic callus production. Maji *et al.* (2002) ^[63] reported that seedlings that varied in 2,4-D medium had their length and weight reduced. As the concentration of 2,4-D increases, values of seedlings length and weight raised in them decreased. But seeds in contact with 2,4-D containing medium levels of the solution gradually increased from 0.0 to 2.0 mg.L⁻¹, but declined gradually at higher levels from 5.0 mg.L⁻¹.

It was studied the effect of plant growth regulators Ivin, Methyur and Kamethur on increasing the efficiency of miniature rose (*Rosa mini* L.) organogenesis in vitro. The growth regulatory activity of plant growth regulators Ivin, Methyur and Kamethur was compared with the activity of plant hormone auxin IAA. It was shown that the effect of synthetic plant growth regulators Ivin, Methyur and Kamethur used at concentrations of 10⁻⁵M, 10⁻⁶M, 10⁻⁷M per 1 liter of MS (Murashige and Skoog) medium on the

organogenesis of shoots and roots of miniature rose (*Rosa mini* L.) in vitro is similar or higher than the effect of the plant hormone auxin IAA used at the same concentrations. The synthetic plant growth regulators showed the highest effect on the organogenesis of shoots and roots of miniature rose

(*Rosa mini* L.) in vitro when used in concentrations: Ivin at concentrations of $10^{-5}M$ and $10^{-6}M$, Kamethur at concentrations of $10^{-5}M$ and $10^{-6}M$, Methyur at concentrations of $10^{-5}M$ and $10^{-7}M$ (Fig.2) (Tsygankova *et al.*, 2023) ^[108].

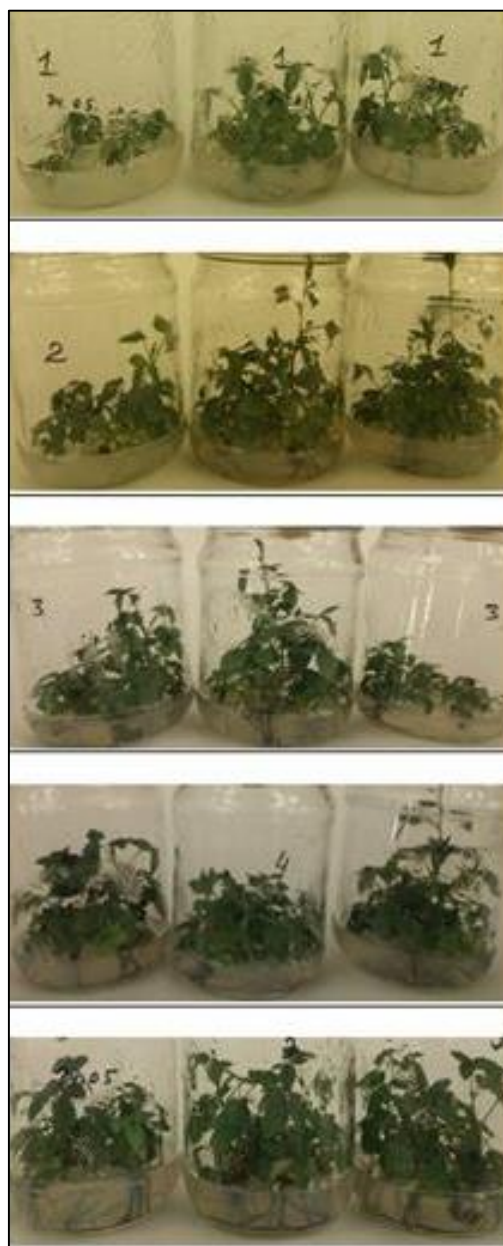


Fig 2: Organogenesis of shoots and roots of miniature rose (*Rosa mini* L.) in vitro, measured on the 28th day of cultivation, (A): 1- control hormone-free MS medium, 2 - MS medium containing Ivin at a concentration of $10^{-6}M$, 3 - MS medium containing Kamethur at a concentration of $10^{-6}M$, 4 - MS medium containing Methyur at a concentration of $10^{-6}M$, 5 - MS medium containing plant hormone auxin IAA at a concentration of $10^{-6}M$

Auxins stimulate cell elongation and influence a host of other developmental responses, such as root initiation, vascular differentiation, tropic responses, apical dominance and the development of auxiliary buds, flowers and fruits. 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, flowers and fruits (Daphne *et al.*, 2005) ^[21]. IAA, IBA and 2,4-dichlorophenoxy acetic acid (2,4-D) as well as picloram are often added to nutrient media. The naturally occurring auxin, IAA, is added in a concentration of 0.001 to 10 mg.mL⁻¹

¹. Pierik (1987) ^[81] showed the influence of high concentration of a weak auxin compared to that of a low concentration of a strong auxin. Results showed more adventitious root formation of NAA (1 mg L^{-1}) and IAA (5 mg L^{-1}) in *Gerbera jamesonii* shoot cutting. Many reports illustrate that plant growth regulators, especially auxins, have striking effects on nicotine accumulation in tobacco tissue cultures. A synthetic auxin, 2,4-D, was inhibitory to nicotine production in *Nicotiana tabacum* callus cultures at concentrations higher than 10^{-6} or $10^{-5} M$, depending on callus lines (Lockwood and Essa, 1984) ^[61]. On the other hand, indolyl-3-acetic acid (IAA) did

not inhibit it at a concentration range between 10^{-6} and 10^{-5} M (Ogino *et al.*, 1978) [77]. Takahashi and Yamada (1973) [104] found that 2,4-D at a lower concentration (10^{-8} M) stimulated nicotine production, and IAA at higher concentrations (10^{-4} , 10^{-3} M) inhibited it in *N. tabacum* cv. Bright Yellow callus cultures. These findings suggest that 2,4-D does not differ from IAA in its effect on nicotine production. Another auxin, naphthaleneacetic acid (NAA), was shown to have a narrow concentration range (0.15–0.2 ppm) optimal to nicotine production in *N. tabacum* cv. Bright Yellow callus (Ohta *et al.*, 1978a) [78]. Nicotine productivity under auxin conditions that are stimulatory or inhibitory to nicotine production is reversible, provided that a callus line has the potential to synthesize the compound (Shiio and Ohta, 1973a) [96].

Seed priming of GA₃ at 100ppm and NAA at 100ppm was an effective treatment to increase mung bean growth, photosynthetic pigments, metabolic activities, yield, yield components, and chemical composition of the yielded seeds as low input technology for improving the yield of mung bean.

Pre-treatment (priming) of two red bean (D81083 and KS31169) seeds with growth regulators including salicylic acid (SA) and naphthalene acetic acid (NAA) at four levels (P0:control, distilled water; P1: 0.5 m mol L⁻¹ NAA; P2: 0.7 m mol L⁻¹ SA; P3: combination of SA and NAA hormones at rates of 0.5 and 0.7 m mol L⁻¹) were studied. The results showed that the effect of line in all of the characteristics and seed priming with growth regulators in all of the characteristics except to harvest index were significant. The highest number of grains per pod and biological yield (14602 kg ha⁻¹) due to growth regulator application was obtained from line D81083 in 0.5 m mol L⁻¹ NAA. Generally, the use of growth regulators as a seed pre-treatment increased the yield and yield components of red beans (Nandan *et al.*, 2021) [72].

Seed priming with IAAs (1 ppm) enhanced the seedling establishment of *Bouteloua gracilis* (Roohi and Jameson, 1991) [86], and in wheatgrass (*Agropyron elongatum*), seeds priming with IAAs at 50 ppm improved tolerance to drought stress by enhancing antioxidant enzyme activities such as catalase (CAT), superoxide dismutase (SOD), and peroxidase (POD) (Eisvand *et al.*, 2010) [27]. Under salinity stress, wheat seeds priming with IAAs (100, 150, and 200 mg L⁻¹) regulated hormonal homeostasis, which enhanced the CO₂ assimilation rate and ultimately resulted in increased grain yield (Iqbal and Ashraf, 2007) [45]. Also, seed priming with IAAs improved the germination and growth of different species, such as rice (*Oryza Sativa*) and pigeon pea (*Cajanus cajan*), under arsenic or cadmium (Cd) stress (Sneideris *et al.*, 2015; Fahad *et al.*, 2015) [100, 28].

An attempt has been made to elaborate the subject with emphasis on important plant growth regulators viz. auxins, cytokinins, Gibberellins and abscisic acid and others. Some environmental stresses which happen to be usually in the way of crop maturity also been discussed. It is revealed the plant growth regulators when applied in proper stage of growth and concentration influenced positively the yield components and yield of soybean (Basuchaudhuri, 2017; Singh *et al.*, 2018) [12, 98].

In direct seeded rice (DSR) GA₃ at 100 ppm and kinetin at 10 ppm effectively improved the emergence (Lee *et al.*, 1999) [57]. Seed soaking with 75 ppm NAA or IAA leads to higher plant dry matter (Watanabe *et al.*, 2001) [115]. NAA might

have enhanced the plant N-content and N being a constituent of phospho-nucleotide helps in cell division and expansion (Kalita *et al.*, 1995) [51]. IBA also increased the number of adventitious roots and root dry-weight in rice seedlings which is highly desirable for DSR rice for better anchorage and ability to absorb nutrients (Wahyuni *et al.*, 2003) [113].

It is important to consider how to improve the nitrogenase activity to increase soybean yield. As early as the 1980s, some researchers found that host plants could secrete flavonoids when infected by rhizobia, which can induce the synthesis and release of nod factors (NFs) in the rhizobia.

Heatin (Jia *et al.*, 2016) [49], which comprises a small aromatic compound and 1-iminomethyl-2-naphthol as a pharmacophore (woude *et al.*, 2021) [110]. Utilizing a chemical proteomics approach, the NITRILASE1-subfamily of auxin biosynthesis enzymes were identified as molecular targets of Heatin. In vitro experiments demonstrated that Heatin inhibits the enzymatic activity of the NIT1-subfamily, resulting in the accumulation of its substrate, indole-3-acetonitrile, in vivo.

Cytokinins are phytohormones synthesized in meristematic organs and tissues (Rademacher, 2015) [83]. Cytokinins regulate diverse physiological and biochemical processes in multiple plant organs, cell proliferation and differentiation, and plant response to stress conditions. The lack of cytokinin may halt the cell cycle and affect cellular activities (George *et al.*, 2008) [31] due to its cardinal role in plant cell division by directly regulating protein synthesis during mitosis. Cytokinins promote root growth, shoot development from internodes, chloroplast maturity, initiate callus formation (Carrow and Duncan, 2011; George *et al.*, 2008) [14, 31] and stimulate plant responses to diverse biotic and abiotic stresses as well as nutrients by preventing cell degeneration, protein synthesis signaling, and augmenting protective enzymes (Carrow and Duncan, 2011) [14]. Exogenous cytokinin increased pod setting by inhibiting flower abortion in soybean and lupin and ultimately increased yield (Najafian *et al.*, 2009) [71]. Dietrich *et al.* (1995) [24] reported that the exogenous application of cytokinin to maize plants during pollination increased the number of kernels and the total kernel weight per ear by reducing apical kernel abortion. Benzyladenine (BA) application stimulated cell division and elongation, increased flower production, and decreased flower drop (Krug *et al.*, 2006) [55]. Daily application of kinetin (Kn; for 5 d starting from 2 d post-anthesis) increased cell division and grain weight of rice (Yang *et al.*, 2003) [118]. Cytokinins are often used to stimulate growth and development, zeatin, kinetin, BAP, 2, P and pyranilbenzyladenine (PBA) being common. They usually promote cell division, especially if added together with an auxin. In high concentration (1 to 10 mg/ml), they can induce adventitious shoot formation, but root formation is generally inhibited. They promote auxiliary shoot formation by decreasing apical dormancy (Slater *et al.*, 2005) [90]. A compound exhibits strong efficacy in vivo activity that against drought and cold stress with wheat, corn and canola. Nisler *et al.* (2018) [76] have synthesized novel urea derivatives, named ASES 49.88. In vivo experiments have demonstrated that ASES exhibit anti-aging properties in *A. thaliana* and enhance salt tolerance in wheat. Notably, ASES have been found to be particularly effective in inhibiting the degradation of chlorophyll in wheat. However, the molecular target of ASES remains unidentified. Oh and Hoshi (2019) [79] synthesized novel pyrazole derivatives, that elicit has a triple response in *Arabidopsis* seedlings and impede root

growth.

The application of CKs to cotton seedlings increased cotton yield by 5–10%. CKs play an important role in plant pathogenesis, and CK application induced resistance against *Pseudomonas syringae* in *Arabidopsis thaliana* (Choi *et al.*, 2010; Grobkinsky *et al.*, 2011) [17, 34] and *Nicotiana tabacum* (Grobkinsky *et al.*, 2011) [34]. CKs may act as a biological agent to control diseases. For instance, *Pseudomonas fluorescens* G20-18 produces CKs, which controls *Pseudomonas syringae* infection in *Arabidopsis* and enhances biomass yield (Grobkinsky *et al.*, 2016) [35]. The exogenous application of CKs can mitigate the abiotic stresses on crop plants, which ultimately results in increased growth, development, and yield. Likewise, supplementation of CKs also reduces salinity stress in plants (Ha *et al.*, 2012; Taiz *et al.*, 2015) [35, 105], and it increases starch accumulation in salt-stressed rice plants (Javid *et al.*, 2011) [47]. In addition, exogenously applied CKs increased net C-assimilation, net photosynthesis, and dry matter accumulation in *Epipremnum aureum*, which resulted in enhanced plant growth (Di Benedetto *et al.*, 2015a,b) [25, 26]. However, Zahir *et al.* (2001) [119] reported that exogenous application of CKs significantly increased the growth and yield of rice.

Seed priming with CKs or a combination of CKs and other plant hormones has resulted in the mitigation of abiotic stresses in various plant species. Priming with CKs enhances chlorophyll (Chl) formation and biomass accumulation in plants, and it increases photosynthetic rate, promotes membrane stability, and maintains stable ionic levels. It has been reported that wheat seeds priming with kinetin (100 mg L⁻¹, 150 mg L⁻¹, and 200 mg L⁻¹) enhanced germination and tolerance against salt by decreasing ABA and increasing IAA concentrations (Iqbal *et al.*, 2006) [44]. Likewise, Mangena (2020) [64] reported that soybean seed priming with CKs (Benzyl adenine; 4.87 mg L⁻¹) increased soybean root biomass, flowering, and fruiting under drought stress. Priming of aged groundnut (*Arachis hypogaea* L.) seeds with CKs (150 ppm) enhanced germination and seedling indices by enhancing antioxidant enzyme activities and decreasing oxidative damage (Sepehri and Rouhi, 2016) [94].

Herbicides

The synthetic auxins include the following herbicide families: benzoic acids, phenoxycarboxylic acids, pyridine carboxylic acids, and quinoline carboxylic acids that act similar to that of endogenous plant auxin. Auxin Transport Inhibitors such as diflufenzopyr, however, inhibit the movement of auxinic compounds out of cells. Consequently, when combined with a synthetic auxin herbicide such as dicamba, the dicamba can move into the cells but cannot move back out of the cell, thus maintaining a greater concentration of the auxinic herbicide within the cell. Diflufenzopyr has minor herbicide activity when applied alone but enhances the activity of auxinic herbicides.

Injury Symptoms: Most auxin herbicides cause similar injury symptoms, but symptom intensity and appearance depend on the herbicide, level of exposure, growth stage, crops, and environmental conditions. In general, trees and shrubs are less sensitive than susceptible annual plants. In addition, younger plants are more susceptible to Auxin Inhibitors than mature plants. Symptoms may range from slight, at low exposure, to severe or death from high levels of exposure. Initial symptoms are twisting, leaf curling, and cupping, which may occur within hours of exposure. Leaves

that are not fully expanded at the time of exposure may be stunted and distorted. A few days after exposure, general chlorosis may develop at high exposure levels. Leaves will drop and shoot tips may die, followed by stem dieback in trees and vines. Growth may resume depending on the level of exposure. Re growth is sometimes limited to buds on the lower part of the plants. Re growth may exhibit severe shoot and petiole twisting, leaf cupping, stunting, curling, strapping, feathering, roughness, crinkling of the leaf surface, vein discoloration, and fingering of the leaf margins. Auxin herbicides may inhibit inter vein tissue growth making veins appear to be joined together and extended to form finger-like projections. In trees, ornamental shrubs, and grapevines, symptoms may continue to appear until the end of the growing season. In grapevines, 2,4-D symptoms from high concentrations may continue to appear in the second year after exposure. Severely injured grapevines may not recover for two years or more. Furthermore, high concentrations of Auxin Inhibitors may cause stem cracking or dark reddish coloring. Stem elongation of plants may be enhanced (at low concentrations) or inhibited (at high concentrations) by growth Auxin Inhibitors.

Injury from high concentrations of Auxin Inhibitors may change fruit size, shape, and appearance or cause abortion of fruits. Slight auxin herbicide symptoms, however, may have no effect on fruit maturity. Exposure to high concentrations of auxin herbicides may also delay fruit ripening when plants are severely affected. For example, delayed maturity from exposure to high 2,4-D concentrations may exist in a grapevine for one to three years before normal ripening returns (Heitshusen, 2022) [38].

Metabolomics and Genetics

Indole-3-acetic acid (IAA) is the basic and most abundant auxin present in plants. Auxins are chemical compounds with aromatic ring and a carboxylic acid group. Tryptophan is the precursor of auxin biosynthesis in plants. The synthetic auxins, 2,4-D and NAA are often converted, after uptake into plant tissues, to conjugates, mainly glucosyl esters (Barendse *et al.*, 1987; Klems *et al.*, 1998) [10, 54]. This reversible conjugation may regulate levels of free active substances.

Now several genes are known, coding for both putative carriers and for the NPA-binding site. Typical representatives of these genes, AUX1 (coding for the putative auxin uptake carrier), PIN gene family (coding for the putative auxin efflux carriers, expressed in different tissues and organs) and others have been identified in *Arabidopsis thaliana* (Morris *et al.*, 2004) [69].

N-terminal signal peptide for transit into the endoplasmic reticulum, one glycosylation site and the C-terminal KDEL (Lys-Asp-Glu-Leu) sequence for retention in the lumen of endoplasmic reticulum. The crystal structure of ABP1 and its interaction with auxin was described by Woo *et al.*, (2002) [117]. Another membrane-associated ABP, showing specificity very similar to that of the maize ABP1, was detected in tobacco cells culture.

Auxin triggers the signal responses directly through stimulation or inhibition of the expression of genes. It activates different families of primary response genes like glutathione S-transferases, auxin homeostasis proteins like GH3 and the Aux/IAA repressors for transcription.

Receptor-like auxin-binding proteins have been identified and characterised by various techniques (traditional ligand-binding studies, photoaffinity labelling and genetic

approaches) as recently reviewed by Napier *et al.*, (2002), Zazimalova and Napier (2003) ^[120], Hagen *et al.*, (2004) ^[73, 121, 36].

There are some candidates for true auxin receptors, especially ZmABP1, i.e. *Zea mays* Auxin-Binding Protein 1, the major auxin-binding protein from maize membranes. This protein exists in the form of a dimer of 22-kDa subunits. It has been purified by several methods and its primary structure was deduced from cDNA clones. Additionally, several other genes encoding this auxin-binding protein (ABP1) have been sequenced from other plants, *Arabidopsis thaliana* and other dicots including tobacco. All homologues share a common primary amino acid sequence containing an N-terminal signal peptide for transit into the endoplasmic reticulum, one glycosylation site and the C-terminal KDEL (Lys-Asp-Glu-Leu) sequence for retention in the lumen of endoplasmic reticulum. The crystal structure of ABP1 and its interaction with auxin was described by Woo *et al.*, (2002) ^[117]. Another membrane-associated ABP, showing specificity very similar to that of the maize ABP1, was detected in tobacco cells cultured *in vitro*.

ARE mediated transcription of auxin genes are initiated by auxin responsive factors (ARF). This transcription is then inhibited by binding of AUX/IAA protein complex. In absence of auxin, AUX/IAA protein complex binds and suppress transcriptional activity of ARFs. When auxin is present it forms a molecular complex between TIR1 protein and AUX/IAA, which results ubiquitine mediated proteolysis of AUX/IAA. ARF are then free to bind to promoter and carry out transcription.

The CKs are key hormones often considered ABA antagonists and auxin antagonists/synergists in various processes in plants responsible for plant growth, development, and tolerance against different abiotic stresses (Pospíšilová, 2003; Danilova *et al.*, 2016) ^[82, 20]. Besides,

other hormonal pathways (e.g., ABA), CKs are activated when a plant is exposed to salt stress. By interacting with other plant hormones like auxins and ABA, CKs can considerably increase salt stress tolerance (Fig.3.) (Iqbal *et al.*, 2006) ^[44].

Plant endogenous CKs are adenine derivatives with either isoprenoid or aromatic side chains found in plants at a lower abundance (Sakakibara, 2006) ^[91]. The isoprenoid CKs can be distinguished as isopentenyl adenine (iP), trans-zeatin (tZ), cis-zeatin (cZ), or dihydrozeatin-type derivatives according to the hydroxylation and reduction of the side chain. The rate-limiting step of isoprenoid CK biosynthesis is catalyzed by isopentenyl transferase (IPTs). Rivero *et al.* (2010) ^[85] generated transgenic tobacco carrying an *Agrobacterium tumefaciens* isopentenyl transferase gene (IPT). Similarly, the promoter of a senescence-associated receptor protein kinase (SARK) gene, from *Phaseolus vulgaris*, was constructed in front of the IPT gene. These transgenic plants (PSARK::IPT) are more tolerant of drought-induced leaf senescence, which results in a remarkable level of water stress tolerance. During water stress, a greater reduction in photosynthesis occurs in susceptible plants than the stress-tolerant transgenic plants due to the photosynthetic apparatus being degraded in susceptible plants under low photosynthesis conditions. Therefore, CKs may protect photosynthetic processes contributing to the stress tolerance in the transgenic plants (Rivero *et al.*, 2010) ^[85]. In addition, recent studies on CK signaling have suggested that this hormone is indeed involved in plant salt stress responses. Synthetic CKs reverse the plants' drought-induced alterations and allow normal growth and developmental activities. Plenty of research findings suggest that CKs support normal growth and development under osmotic stresses and improve plants' drought tolerance ability.

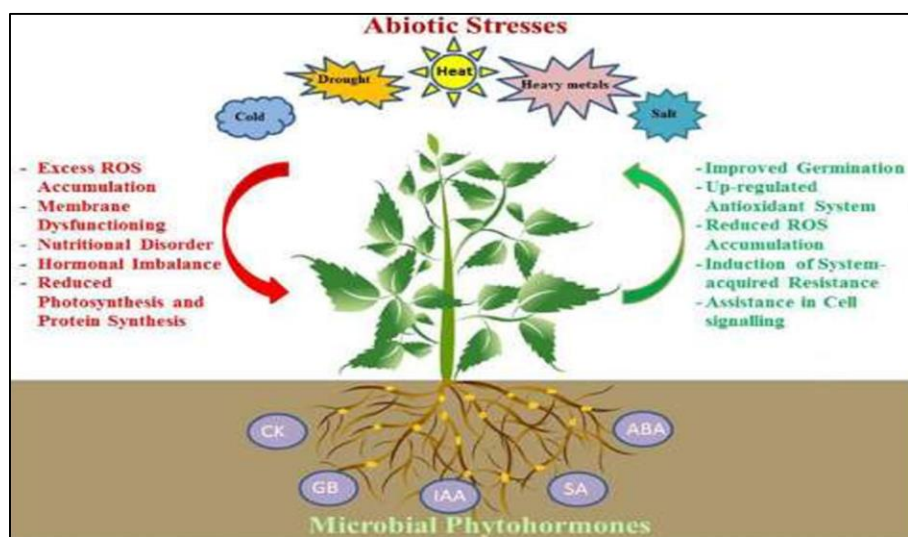


Fig 3: Mechanisms in microbial phytohormone-mediated plant stress tolerance. Several root-associated microbes produce CK, GA, IAA, SA, and ABA, which help plants to withstand stresses

The cooperation between gene regulatory networks (GRNs) and phytohormones in cell development and morphogenesis represents a cornerstone of plant developmental biology. The interaction between these two biological systems governs many aspects of plant growth and differentiation, helping to shape the architecture of plants in response to various environmental and internal signals (Van den Broeck *et al.*,

2020) ^[111]. GRNs consist of transcription factors (TFs), target genes, and other regulatory elements that coordinate the expression of genes in response to developmental cues and refer to the complex web of gene interactions that determine when and where individual genes are activated or repressed (Huang and Kauffman, 2009) ^[43].

The stem cell population in the shoot apical meristem (SAM)

is maintained by cytokinin. The two distinct cytokinin dependent pathways, CLAVATA (CLV)-dependent and CLV independent, control WUS levels (Gordon *et al.*, 2009). High levels of cytokinin signaling in *Arabidopsis* cause ectopic WUSCHEL (WUS) expression and influence neighboring cells' stem cell fate (Lee *et al.*, 2019) [56]. WUS directly represses the transcription of several two-component ARABIDOPSIS RESPONSE REGULATOR genes (ARR5, ARR6, ARR7, and ARR15), which act in the negative-feedback loop of cytokinin signaling (Leibfried *et al.*, 2005) [58]. These data imply that cytokinin is adequate for the induction of WUS and the determination of stem cell fate. GRNs determine the expression of genes that are vital for the cell cycle in response to these hormonal signals. As a noncanonical AUXIN RESPONSE FACTOR (ARF), ARF3/ETTIN (ETT) mediates auxin responses to orchestrate multiple developmental processes during the reproductive phase. GRNs respond to hormone cues by regulating cell cycle genes. The

ARF3/ETT modulates auxin responses to coordinate reproductive development, according to recent findings by Fu *et al.*, 2000 [30]. It is stated that ARF3 maintains dynamic SAM non-autonomously. Regarding the temporal aspect, the term "cell differentiation" may also refer to the development of the unicellular organism for the origin of different growth stages and division cycles or the phases in which this cycle is interrupted. These antagonistic interactions between CLV and WUS are tightly integrated with hormone function, demonstrating the role in cell division.

The chosen genes encode products associated with cytokinin metabolism (TaIPT5, TaIPT8, TaCKX3, TaCKX4, TaCKX6, TaGLU1, TaGLU2) and response (TaRR1, TaRR4, TaRR9), antioxidant defense (TaCAT, TaSOD) and senescence (TaSAG3, TaSAG5, and TaSAG8) in wheat.

GRNs and phytohormones have a hand in differentiating cells into various tissue types. For instance, the balance between auxin and cytokinin determines whether a cell differentiates into root or shoot tissue. Plants and other eukaryotic use sophisticated gene expression processes to regulate development, environmental and cellular homeostasis. Plants generate new organs throughout their lifespan due to the pluripotent stem cell population, unlike mammals. It is known that cell differentiation relies on the TFs, form the GRNs with the basic cofactors and Post translational regulators for specialized developmental activities (Kaufmann and Chen, 2017) [52]. The activities around DNA are controlled by hormones and chemicals, which decide what is transcribed and what is ignored. The body and nearby cells determine the variables that affect a cell from birth to death (Satterlee *et al.*, 2020) [92]. For instance, the pancreas or thyroid may release hormones involved in cellular growth. These TFs directly impact the proteins that transcribe DNA, ultimately producing functioning proteins and more cells. However, when cells congress, they build up communication with one another so there is no more space. Thus, this interaction process between hormones and GRNs on cell differentiation is involved in plant growth and development, including meristem function, vascular development, stress responses, and senescence (Satterlee *et al.*, 2020) [92].

Hormones such as gibberellins promote cell elongation. GRNs will mediate the expression of genes vital for cellular elongation in response to gibberellin signals. Jasmonic acid (JA) promotes potato tuber formation, reducing the leaf primordial length, meristem enlargement, and cell expansion

(Naturales, 2003) [74]. Proper root development requires ROS homeostasis, which regulates cell proliferation and differentiation at root tips. Plant roots collect significant quantities of ROS; when this unbalances, the plant cannot adapt to environmental changes and dies. Moreover, ROS controls cell expansion and cell processes such as root hair formation and lateral root development (Mase and Tsukagoshi, 2021) [65]. Auxin is involved in cell enlargement and proliferation. During the process of tuberization, auxin in potato plant dynamics affects tuber initiation by stimulating its biomass and increasing tuberization (Aksenova *et al.*, 2012) [4]. IAA transport from shoot to root and the stolon is necessary for tuberization. StYUC-like 1 expression increases at stolon tips during tuberization.

Plants possess a small group of pluripotent stem cells in their meristems. The shoot apical meristem (SAM) plays a key role in forming all of the aerial structures of plants, including floral meristems (FMs). The FMs subsequently give rise to the floral organs containing reproductive structures. Studies in the past few decades have revealed the importance of transcription factors and secreted peptides in meristem activity using the model plant *Arabidopsis thaliana*. Recent advances in genomic, transcriptomic, imaging, and modeling technologies have allowed us to explore the interplay between transcription factors, secreted peptides, and plant hormones. Two different classes of plant hormones, cytokinins and auxins, and their interaction are particularly important for controlling SAM and FM development.

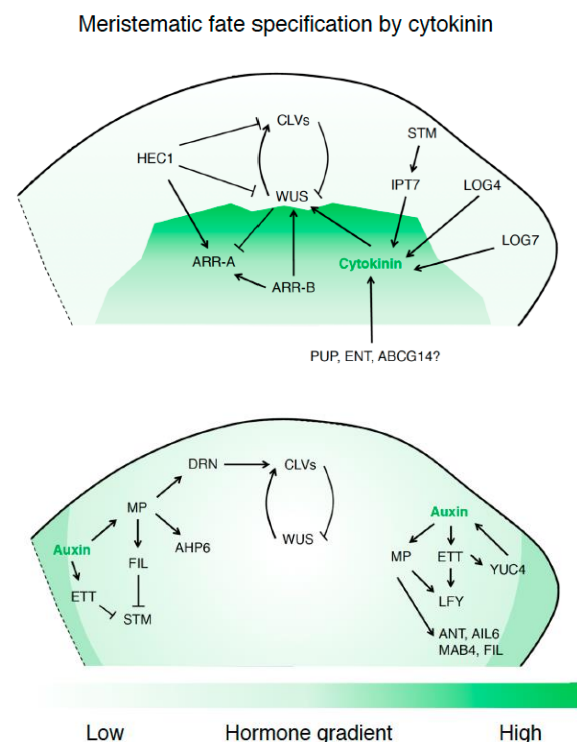


Fig 4: Two different classes of plant hormones, cytokinins and auxins, and their interaction are particularly important for controlling SAM and FM development

The robust establishment of the SAM and FM is primarily controlled by conserved secreted peptides and master transcriptional regulators. In the past five years, plant stem cell researchers have identified various interactions between these key factors and phytohormones using a candidate gene approach or omics data. Phytohormones help determine when

and where these factors function. The spatiotemporal fine-tuning of these factors by phytohormones appears to be regulated by complex multilayered networks of hormonal components, since hormones affect not only transcription, but also the epigenetic state, protein stability/protein–protein interactions, and metabolic rates of meristematic cells.

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