Phytohormones At a Glance

Faseela P.

Post Graduate Department of Botany
Korambayil Ahamed Haji Memorial Unity Women's College,
Manjeri, Narukara (PO), PIN: 676122, Malappuram (DT), Kerala, India.

Edited by:
Faseela P.
Assistant Professor
Post Graduate Department of Botany
Korambayil Ahamed Haji Memorial Unity Women's College, Manjeri, Narukara (PO),
PIN: 676122, Malappuram (DT), Kerala, India.
Email: faseela8888@gmail.com

First Edition: March 2024
ISBN: 978-93-6013-017-6

Published by:
Faseela P.
Assistant Professor
Post Graduate Department of Botany
Korambayil Ahamed Haji Memorial Unity Women's College, Manjeri, Narukara (PO),
PIN: 676122, Malappuram (DT), Kerala, India.

Price: ₹750/-
Date of publication: March 2024
Printed at: Apex Digital Services, Manjeri, Malappuram (DT), Kerala, India.

Preface

Phytohormones, a cornerstone of plant biology that intricately governs growth, development and responses to environmental cues. This comprehensive compilation delves deeply into their structures, biosynthesis, signal transduction and physiological functions, unveiling the profound impact they wield on plant survival and adaptation. Phytohormones, also known as plant hormones, are a diverse group of small molecules that act as chemical messengers, regulating a myriad of processes essential for plant survival and adaptation. From seed germination and organogenesis to stress responses and defence mechanisms, phytohormones serve as master regulators, orchestrating the intricate dance of plant growth and development.

The introductory part of the book provides a comprehensive overview, detailing the diverse types and physiological functions of these essential chemical messengers in plants, followed by the detailed structures, biosynthetic pathways, signal transduction mechanisms and pivotal functions of key phytohormones, including auxins, cytokinins, gibberellins, abscisic acid, ethylene, jasmonic acid and brassinosteroids. This book elucidates the intricacies of plant hormone biology and inspires further inquiry and innovation in the field. By fostering a deeper comprehension of phytohormonal regulation, this compilation serves as a catalyst for pioneering research and discovery in the dynamic field of plant hormone biology.

Faseela P.

In memory of Korambayil Ahamed Haji Sahib

The founder, Korambayil Ahamed Haji Memorial Unity Women's College, and the visionary leader who could see beyond the current situation and imagine a better future which could inspired others to dream big and work hard to turn those dreams in to reality.

Contents

Plant Hormones: An Overview

Akhila Sen

PG Department of Botany, Mar Athanasius College, Kothamangalam, Ernakulam, 686666, Kerala, India
Email: akakhila12@gmail.com

Abstract

Plant hormones, also known as phytohormones, are chemical messengers that regulate various aspects of plant growth, development, and responses to environmental stimuli. This abstract provides an overview of the key roles and functions of phytohormones in plant physiology. These signaling molecules orchestrate processes such as seed germination, root and shoot growth, flowering, fruit development, and stress responses. Classified into several groups including auxins, cytokinins, gibberellins, abscisic acid, ethylene, jasmonic acid and brassinosteroids, phytohormones exhibit complex interactions and synergies, finely tuning plant growth and adaptation to changing conditions. Understanding phytohormone dynamics is crucial for optimizing agricultural practices and enhancing crop productivity in a sustainable manner.

Key words: Abiotic stress, Biotic stress, Phytohormone, Signaling molecule.

1. Introduction

Like all living things, plants face a variety of environmental difficulties. Abiotic stresses come from non-living sources including, drought, salt, and temperature swings, whereas biotic stresses are caused by interactions with other living things like pathogens, pests, and herbivores. Plants need a sophisticated network of signaling pathways to respond to

these challenges, and plant hormones are essential to this process. Phytohormones are signal molecules produced by plants in very low amounts. Originally considered as regulators of growth, development, and physiological processes similar to animal hormones, it is now understood that they play a role not only in endocrine signaling but also in autocrine and paracrine signaling to facilitate both local and systemic responses to environmental stimuli (Davies 2010). Plant hormones such as auxins, cytokinins, gibberellins, and brassinosteroids play a significant role in regulating plant development and growth. Abscisic acid is known for its role as an abiotic stress hormone, while ethylene, jasmonic acid, and salicylic acid are key players in regulating plant immune responses. Plant hormones interact in intricate networks to maintain a balance between developmental and environmental responses. The relative ratios of these hormones are as crucial as their absolute concentrations. The molecular processes that control these hormonal networks are mostly unknown (Fahad et al. 2015).

2. Class of phytohormones

Phytohormones, or plant hormones, are signaling molecules that regulate various physiological processes in plants, influencing growth, development, and responses to environmental stimuli. The major types of phytohormones include auxins, cytokinins, gibberellins, abscisic acid, ethylene, jasmonic acid, and brassinosteroids. These phytohormones interact with each other and with environmental cues to finely regulate plant growth, development, and responses to biotic and abiotic stresses. Understanding their roles and interactions is essential for optimizing agricultural practices, enhancing crop productivity, and improving plant resilience (Nadeem et al. 2016).

Auxins, such as indole-3-acetic acid (IAA), are fundamental for controlling cell elongation, apical dominance, and tropisms like phototropism and gravitropism. They regulate processes like root initiation, fruit development, and vascular tissue differentiation. Cytokinins, like zeatin, promote cell division and differentiation, influencing shoot and root development, chloroplast formation, and delaying senescence. They interact with auxins to regulate plant growth and development. Gibberellins are essential for stem elongation, seed germination, and flowering. They regulate various developmental processes, including fruit growth and development. Abscisic acid plays a crucial role in plant responses to environmental stresses, such as drought, salinity, and cold. It induces stomatal closure to reduce water loss, promotes seed dormancy, and regulates gene expression under stress conditions (Zhao et al. 2021).

Ethylene is involved in various physiological processes, including fruit ripening, leaf abscission, senescence, and response to mechanical stress. It also plays a role in root development and gravitropism. Jasmonic acid is a key regulator of plant responses to biotic stresses, such as herbivory and pathogen attack. It induces the synthesis of defence-related compounds like toxins and volatile organic compounds, contributing to plant defence mechanisms. Moreover, brassinosteroids promote cell elongation, cell division, and vascular differentiation. They are involved in various developmental processes, including seed germination, pollen tube growth, and stress responses (Manghwar et al. 2022).

3. Functions of plant hormones

Plant hormones control all the growth and development activities like cell division, enlargement, flowering, seed formation, dormancy and

abscission. Based on their action, plant hormones are categorised into plant growth promoters and plant growth inhibitors. Phytohormones in plants regulate growth, development, and responses to environmental stimuli. Auxins control cell elongation, root initiation, and tropic responses. Cytokinins promote cell division, delay senescence, and regulate bud growth. Gibberellins regulate stem elongation, seed germination, and flowering. Abscisic acid mediates stress responses, seed dormancy, and stomatal closure. Ethylene coordinates fruit ripening, leaf senescence, and responses to stress. Brassinosteroids stimulate cell elongation, differentiation, and stress tolerance. Jasmonic acid regulate defence against herbivores, pathogens, and stress. Salicylic acid activates defence mechanisms against pathogens. These hormones interact to orchestrate growth, development, and adaptation in plants, ensuring their survival and reproduction (Vos et al. 2013).

4. Biotic stress and phytohormones

Plant hormones are responsible for mediating defence responses that are triggered in response to biotic stressors, such as pathogen infections or herbivore feeding. Salicylic acid and jasmonic acid two important hormones that play a role in the body's reaction to biotic stressors. While jasmonic acid is engaged in defence against herbivores and necrotrophic pathogens, salicylic acid predominantly controls defence against biotrophic pathogens. These hormones operate as mediators of signaling pathways that frequently interact and crosstalk with one another to provide a coordinated defence response. For example, depending on the kind of stress and the stage of the plant's development, the salicylic acid and jasmonic acid pathways may oppose or complement one another (Vos et al. 2013).

Plants must deal with biotic elements like harmful microorganisms and herbivorous insects that provide them with nutrition. Effective defence against these predators relies on preexisting defences and the activation of their immune system. Preformed non-specific defences consist of physical barriers like cuticles, cell walls, needles, thorns, or trichomes, along with chemical substances (Freeman and Beattie 2008). Plants must deal with biotic elements like pathogenic microorganisms and herbivorous insects throughout their lives, which they use as a source of nutrients. Effective defence against these predators relies on preexisting defences and triggering their immune system. Preformed non-specific defences consist of physical barriers like cuticles, cell walls, needles, thorns, or trichomes, along with chemical substances (Freeman and Beattie 2008). The main components of plant defence signaling involve salicylic acid, jasmonic acid, and ethylene which control distinct reactions to either biotrophic or necrotrophic diseases (Pieterse et al. 2009). The central backbone is significantly influenced by the antagonism between jasmonic acid and salicylic acid, with ethylene playing a supplementary role as a modulator. These three hormones play a vital role in regulating plant immunity and are crucial for certain resistance mechanisms. Salicylic acid predominantly mediates systemic acquired resistance, while jasmonic acid and ethylene are engaged in induced systemic resistance (ISR), all of which are considered partial resistance mechanisms (Tsuda and Katagiri 2010).

5. Abiotic stress and phytohormones

In order to help plants adapt and survive, abiotic conditions including drought, salt, and extremely high or low temperatures cause many plant hormones to be produced and signalled. One important hormone that controls reactions to different abiotic stressors is abscisic acid. By inhibiting

transpiration, sealing stomata, and encouraging root development to reach water sources in the soil, abscisic acid aids in the conservation of water by plants. Another hormone that plays a role in the plant's reaction to abiotic stressors including flooding and mechanical injury is ethylene. Numerous physiological processes, such as fruit ripening, stress response, and seed germination, are regulated by ethylene signaling pathways (Kosova et al. 2011). Although each hormone has a unique function in plant stress responses, there is a great deal of interaction and integration between the many hormones signaling pathways. Plants are able to adapt their reactions to the type and severity of stress they face thanks to the complex web of connections. Additionally, plant hormones frequently control the synthesis of osmoprotectants, the generation of antioxidants, and the expression of genes that respond to stress all of which support adaptation and stress tolerance. In conclusion, the intricate systems that plants have evolved to survive and flourish in harsh settings are highlighted by the dynamic interaction of plant hormones in response to biotic and abiotic challenges. Comprehending these hormone-mediated reactions is crucial for formulating tactics to fortify crops and boost agricultural output in the face of worldwide environmental shifts (Großkinsky et al. 2016).

Plant growth is significantly influenced by many environmental cues that might trigger abiotic stress responses, ultimately restricting plant fitness and crop yield. Plants have developed intricate signaling pathways to detect environmental signals and respond effectively to varied stress circumstances through biochemical, physiological, and molecular changes. The plant must effectively manage the conflicting demands and efficiently respond to stress conditions while also minimizing the negative effects of these responses on tissue integrity, photosynthetic capacity, and primary metabolism. Plant

reactions to abiotic stress factors including drought and salinity have been thoroughly researched (Huang et al. 2011), marking an early alert stage where plants are impacted by the stress. Subsequently, an adaptation phase occurs where the homeostasis is adapted to the new growth condition (Kosova et al. 2011).

Abiotic stress conditions can be brought on by heavy metal pollution, heat, cold, humidity, oxidative stress, and drought. Plant reactions to these circumstances are intricate, involving numerous pathways operating at different levels throughout different developmental stages and containing a significant number of signaling molecules. Typical reactions at the cellular level include alterations to cell wall architecture, adjustments to membrane systems, alterations in the cell cycle and division, modified nitrogen and carbohydrate metabolism, and changed hormone synthesis. The induction of stress-inducible genes involved in membrane integrity, the biosynthesis of osmolytes (proline, glycine betaine, sugars), the detoxification of reactive oxygen species (ROS) (glutathione-S-transferase, hydrolases, catalase, superoxide dismutase, ascorbate peroxidase, peroxidases, glutathione reductase, dehydroascorbate reductase, monodehydroascorbate reductase), mRNA binding, and regulatory proteins like transcription factors, (receptor) protein kinases, and proteinases are examples of shared molecular responses (Dos Reis et al. 2012).

A significant amount of crosstalk occurs between the various hormonal pathways (Peleg and Blumwald 2011) that occurs at the level of hormone biosynthesis, degradation, transport, signaling components, particularly the MAPK cascade (Singh and Jwa 2013), as well as their downstream target genes. Hormones play a central role in signaling and subsequent stress responses. Given that plants are sessile organisms that are

frequently exposed to both biotic and abiotic stress-related stimuli simultaneously, it will be crucial to develop experimental strategies that account for these frequently occurring multiple stress scenarios. The intricacy of plant reactions can only be understood by employing this method, which also identifies the areas of intracellular stress signal integration as possible targets for cross-resistance engineering. However, there's a notable lack of information regarding how plants respond when faced with numerous stressors concurrently. Nevertheless, understanding these mechanisms is crucial for effectively enhancing crop resistance to diverse stressors.

6. Conclusion

Phytohormones, vital signaling molecules in plants, comprise auxins, cytokinins, gibberellins, abscisic acid, ethylene, jasmonic acid and brassinosteroids, regulating growth, development, and stress responses. They play crucial roles in bolstering plant resilience against biotic stresses by inducing defence mechanisms against pathogens and pests. Additionally, phytohormones aid in combating abiotic stresses such as drought, salinity, and extreme temperatures by orchestrating physiological and biochemical adjustments. Through intricate crosstalk among hormone pathways, plants dynamically adapt to stressors. Manipulating phytohormone signaling pathways holds promise for developing stress-tolerant crops, ensuring agricultural sustainability amidst challenging environmental conditions.

References

Davies PJ (Ed.) (2010) Plant Hormones: Biosynthesis, Signal Transduction, Action, 3d ed. Springer, Berlin.

Dos Reis SP, Lima AM, de Souza CR (2012) Recent molecular advances on downstream plant responses to abiotic stress. Int J Mol Sci 13: 8628–8647.

Fahad S, Nie L, Chen Y, Wu C, Xiong D, Saud S, Hongyan L, Cui K, Huang J (2015) Crop plant hormones and environmental stress. Sustain Agri Rev 15: 371–400.

Freeman BC, Beattie GA (2008) An overview of plant defenses against pathogens and herbivores. Plant Health Instr doi: 10.1094/PHI-I-2008-0226-0.

Großkinsky DK, van der Graaff E, Roitsch T (2016) Regulation of abiotic and biotic stress responses by plant hormones. In: Collinge DB (ed) Plant Pathogen Resistance Biotechnology. Wiley and Sons, New York, pp 131–154.

Huang GT, Ma SL, Bai LP, Zhang L, Ma H, Jia P, Liu J, Zhong M, Guo ZF (2011) Signal transduction during cold, salt, and drought stresses in plants. Mol Biol Rep 39: 969–987.

Kosova K, Vitamvas P, Prasil IT, Renaut J (2011) Plant proteome changes under abiotic stress - Contribution of proteomics studies to understanding plant stress response. J Proteomics 74: 1301–1322.

Manghwar H, Hussain A, Ali Q, Liu F (2022) Brassinosteroids (BRs) role in plant development and coping with different stresses. Int J Mol Sci 23(3): 1012.

Nadeem SM, Ahmad M, Zahir ZA, Kharal MA (2016) Role of phytohormones in stress tolerance of plants. Plant, Soil and Microbes: Volume 2: Mechanisms and Molecular Interactions, 385–421.

Peleg Z, Blumwald E (2011) Hormone balance and abiotic stress tolerance in crop plants. Curr Opin Plant Biol 14: 290–295.

Pieterse CMJ, Leon-Reyes A, van der Ent S, van Wees SCM (2009) Networking by small-molecule hormones in plant immunity. Nat Chem Biol 5: 308–316.

Singh R, Jwa NS (2013) The rice MAPKK-MAPK interactome: the biological significance of MAPK components in hormone signal transduction. Plant Cell Rep 32: 923–931.

Tsuda K, Katagiri F (2010) Comparing signaling mechanisms engaged in pattern-triggered and effector triggered immunity. Curr Opin Plant Biol 13: 459–465.

Vos IA, Pieterse, CM, Van Wees, SC (2013) Costs and benefits of hormone-regulated plant defences. Plant Pathol 62: 43–55.

Zhao B, Liu Q, Wang B, Yuan F (2021) Roles of phytohormones and their signaling pathways in leaf development and stress responses. J Agric Food Chem 69(12): 3566–3584.

The Dynamics of Auxin in Plant Growth

Hana Backer

PG Department of Botany, Korambayil Ahamed Haji Memorial Unity Women's College
Manjeri, Malappuram, 676122, Kerala, India
Email: hanabacker95@gmail.com

Abstract

Auxin, a paramount plant hormone, holds pivotal importance in orchestrating fundamental aspects of plant growth and development. Its influence spans critical functions such as cell elongation, root and shoot patterning, vascular differentiation, and tropic responses. The significance of auxin lies in its ability to regulate gene expression, thereby governing diverse physiological processes essential for plant life. At the core of auxin signalling is the intricate interplay between the auxin receptor complex TIR1/AFB, the Aux/IAA transcriptional repressors, and the ARF transcription factors. This cascade of events culminates in genome-wide transcriptional responses, dictating the plant's response to environmental stimuli and shaping its architectural framework. The context-specificity of auxin responses, governed by diverse TIR1/AFB co-receptor pairs, underscores its crucial role as a master regulator in the dynamic landscape of plant biology.

Keywords: Auxin, Plant hormone, Cell elongation, Tropism, Signaling.

1. Introduction

Darwin (1880) hypothesized the presence of a signal that was carried from the tip of the coleoptile to the bending regions lower down, at the time when he made his initial observations on the phototropism of grass

coleoptiles. Went in the Netherlands was eventually able to isolate the chemical by diffusion from coleoptile tips into agar blocks after multiple workers further characterized the signal's movement. These blocks, when replaced on the tips of decapitated coleoptiles, stimulated the growth of the decapitated coleoptiles and caused them to bend when placed asymmetrically on these tips. This revealed the presence of a growth-promoting substance that was produced at the tips of the coleoptiles, transported basipetally, and, when dispersed asymmetrically, caused the coleoptile to bend away from the side where the concentration was higher. Went originally gave this material the name *Wuchsstoff*, which was eventually altered to *auxin*. The substance was ultimately determined to be the straightforward chemical indoleacetic acid, or IAA, after several incorrect identifications (Wildman 1997).

The relevance of auxin is highlighted by the fact that it is involved in virtually every cellular decision made during plant development as well as reactions to environmental factors, abiotic stress, and growth tropisms. Auxin biology has advanced significantly in recent years, especially with regard to *Arabidopsis thaliana*, and the elements and mechanisms of auxin sensing and response have been largely clarified. (Abel and Theologis 2010).

2. Biosynthesis

Auxins are low molecular weight organic acids with an aromatic ring and a carboxyl group that must be 0.55 Å apart in order for them to be active. IAA is the most prevalent endogenous auxin and can perform the majority of auxin functions necessary for plant development and environmental response. Only three other naturally occurring chemicals with auxin activity have been identified in plants, namely phenylacetic acid (PAA), 4-chloroindole-3-acetic acid (4-Cl-IAA), and indole-3-butyric acid (IBA).

These molecules complement IAA. They have been found in conjugated and free acid forms (Sauer et al. 2013).

Over the past few years, genetic, biochemical and molecular studies have greatly advanced our understanding of auxin biosynthesis. Indole-3-acetic acid (IAA), plant auxin, is essential for the growth and development of plants. The Trp-dependent (TD) and Trp-independent (TI) pathways are the two main methods suggested for plants to synthesise IAA. Indole-3-acetamide, indole-3-pyruvic acid, tryptamine, and indole-3-acetaldoxime are the four TD routes that have been theorised, identified, and widely researched. Auxin production pathways in some bacteria are remarkably comparable to those in plants, showing conserved biosynthetic mechanisms (Di et al. 2016).

Auxin plays a pivotal role in regulating crucial aspects of plant growth and development, encompassing cell division, elongation, tropisms, apical dominance, senescence, blooming, and stress response (Yang et al. 2014). The three primary auxins in plants are IAA, 4-Cl-IAA, and PAA, with IAA being the most abundant and significant (Simon and Petrasek 2011). Maintaining auxin homeostasis is vital for normal plant development, achieved through the control of auxin transport, production, and conversion. Plant species employ both tryptophan-dependent (TD) and tryptophan-independent (TI) pathways for auxin biosynthesis, including the IAM, IPyA, TAM, and IAOx pathways (Woodward and Bartel 2005; Chandler 2009). Abiotic factors such as temperature and wounding cooperatively regulate free IAA levels, while recent research has identified enzymes and regulators, particularly in the IPyA pathway, contributing to the active regulation of auxin biosynthesis (Reverberi et al. 2005).

2.1 L-Trp biosynthesis pathway in plants

Plant cells synthesize Trp from chorismate via indole-3- glycerol phosphate (IGP) in the chloroplast (Radwanski and Last 1995). This process includes six critical steps. Anthranilate synthase (AS), a rate-limiting step in Trp biosynthesis, is catalysed by the WEI2/ASA1 and WEI7/ASB1 genes in *Arabidopsis thaliana*. AS catalyses the conversion of chorismate to anthranilate. While the β subunit transfers an amino group from glutamine, the α subunit attaches to chorismate and catalyses its aromatization. Anthranilate is converted to 1-(O-carboxylphenylamino)-1-deoxyribulose-5-phosphate, which is also called CdRP by phosphoribosylanthranilate transferase (PAT1) and phosphoribosylanthranilate isomerase (PAI1/2/3). CdRP is then catalyzed by indole glycerol phosphate synthase (IGS) into IGP. IGP is a branch point that can lead to TI auxin synthesis directly from indole (Mano and Nemoto 2012). IGP is converted by the Trp synthase to make trp (Last et al. 1991).

2.2 Trp-dependent IAA biosynthesis pathways

The IAM pipeline, IPyA pathway, IAOx pathway, and TAM pathway are the four currently recognised TD pathways of IAA biosynthesis. Genes involved in the manufacture of Trp derivatives and well-known intermediary metabolites define these pathways.

2.2.1 The indole-3-acetamide (IAM) pathway

The IAM (Indole-3-Acetamide) route, initially identified in *Pseudomonas* and other bacteria associated with plants, involves the conversion of L-Trp to IAM by tryptophan monooxygenase in bacteria

(Casanova et al. 2005). In the subsequent step, indole acetamide hydrolase catalyzes IAM to synthesize IAA (Indole-3-Acetic Acid) (Pollmann et al. 2003). Previously believed exclusive to bacteria, the discovery of IAM's metabolic conversion to IAA in Arabidopsis revealed a plant-centered IAM route, likely distinct from bacterial processes. The enzyme Acylamidohydrolases (AMI1) facilitates the IAM pathway's second step, transforming IAM into IAA. Evidence supporting the significance of the IAM pathway includes its detection in seaweeds, algae, and various plants, along with genetic and molecular studies affirming its presence across the plant kingdom (Lehmann et al. 2010).

2.2.2 The indole-3-pyruvic acid (IPyA) pathway

The IPyA (Indole-3-Pyruvic Acid) route is a common pathway in bacteria, notably in *Enterobacter cloacae* and *Azospirillum brasilense*, fostering plant root growth. In this route, L-Trp is converted to IPyA by tryptophan aminotransferase (AT) in bacteria. Indole-3-pyruvate decarboxylase (IPDC) transforms indole-3-acetaldehyde (IAD) from IPyA, and IAD is oxidized by indole-3-acetaldehyde oxidase (IAO) to produce IAA (Zhao 2014). TAA1, a key enzyme, catalyzes IPyA formation from L-Trp in plants, influencing various aspects of plant growth. TAA1 and YUC regulate IPyA homeostasis, with transcription factors like SHORT-INTERNODES 1/STYLISH 1 (SHI1/STY1) controlling their expression. YUC's mechanism for catalyzing IPyA to IAA involves NADPH and O2 (Stepanova et al. 2008). The IPyA pathway represents the first fully elucidated IAA biosynthesis pathway in plants (Dai et al. 2013).

2.2.3 The tryptamine (TAM) pathway

The role of tryptamine (TAM) in plant growth is debated, despite its similar properties to IAA. Present in species like tomato, rice, Arabidopsis, barley, and pea, TAM's significance is uncertain, as it may exist non-specifically or at levels inconsistent with a discrete signal (Quittenden et al. 2009). TAM is not the primary precursor of IAA, and they arise from separate Trp pools. Tryptophan decarboxylase (TDC) is believed to convert L-Trp into TAM, followed by YUC catalysis to produce hydroxytryptamine (HTAM). IAOx is then synthesized from HTAM, leading to IAA (Zhao et al. 2001). While the TAM pathway's role in IAA biosynthesis is not firmly supported, it remains a potential precursor, with instances of TAM conversion to IAA in specific plant tissues, suggesting a variable existence across plant species and organs (Brumos et al. 2014; Tivendale et al. 2014).

2.2.4 The indole-3-acetaldoxime (IAOx) pathway

Although isolated over 40 years ago, the precise biosynthesis mechanism of IAOx (Indole-3-Acetaldehyde Oxime) remains unknown (Sugawara et al. 2009). In Arabidopsis, IAOx synthesis from L-Trp involves cytochrome P450 enzymes CYP79B2 and CYP79B3. CYP79B2 overexpression leads to an IAA-overproduction phenotype. Studies suggest that both IAM and IAN are downstream intermediates of IAOx, with CYP71A13 converting IAOx to IAN. The conversion of IAN to IAA is unclear, but nitrilases (NITs) may be involved. However, IAOx-dependent IAA production appears non-standard, likely functioning selectively in specific settings or developmental stages, as observed in Arabidopsis but not in tomato, rice, maize, pea, or tobacco (Vorwerk et al. 2001; Nafisi et al. 2007).

2.4 Trp-independent pathway

The TI (Tryptophan-Independent) pathway, documented in maize, *Arabidopsis*, carrot, *Lemna gibba, Nicotiana tabacum*, and tomato, functions alongside the TD (Tryptophan-Dependent) pathway to regulate auxin homeostasis (Epstein et al. 2002). While the TD pathway maintains high IAA levels for cell division, the TI pathway is suggested to sustain basal auxin levels. Developmental stages and tissue types influence the pathways' contributions to auxin content. In plants, both pathways are active, with the TI circuit exhibiting feedback inhibition. Trp's significance in IAA biosynthesis is debated, and an indole-synthesizing TI route has been discovered (Ribnicky et al. 2002). Challenges in identifying TI pathway mutants or intermediate metabolites may arise from the dominance of the TD pathway, metabolite similarity, or the use of unsuitable model plants like Arabidopsis and rice (Wang et al. 2015).

3. Functions of auxin

Plants in the angiosperm family go through various stages of life cycle transitions. For the development of plants, these many transition periods are essential. These changes are influenced by both internal and external cues, such as phytohormones and environmental conditions. An essential component of plant morphogenesis, auxin is a traditional phytohormone that is involved in a number of physiological processes, including phototropism, cell differentiation, cell expansion, floral opening, organ abscission, and seed germination (Wu et al. 2020).

Research has shown that phytohormones generated from chorismate, such as auxin, melatonin and SA, not only have a common precursor but also

are crucial for controlling fruit ripening and growth. They interact with other phytohormones in a spatiotemporal manner to exchange functional and metabolic signals that carefully control the development of climacteric and non-climacteric fruits. It is evident from the variations in dynamics between climacteric and non-climacteric fruits that the reaction of hormones generated from chorismate is very species-specific rather than universal. Moreover, phytohormones generated from chorismate regulate the build-up of bioactive substances, which affects the quality of the fruit (Pérez-Llorca et al. 2019).

3.1 Auxin and stem cell elongation

Auxin plays a pivotal role in promoting rapid cell elongation in plant stems and coleoptiles. This response is marked by a swift and dramatic increase in growth rate, initiated within 10 minutes of auxin application, resulting in a 5–10 times boost in cell length, lasting for hours or days. The process of auxin-induced cell expansion involves two interconnected mechanisms: the extension of the existing cell wall fuelled by turgor-induced stress and the osmotic uptake of water driven by a water potential gradient.

Energy, specifically ATP synthesis, is crucial for auxin-induced growth, as demonstrated by the inhibition of growth within minutes when ATP synthesis is blocked. Auxin-induced development is a tissue response, where all connected cells collectively elongate or not based on shared cell walls. While auxin primarily targets exterior cell layers (collenchyma and epidermis) in dicot stems, it can also influence interior cells in certain situations.

Auxin enhances wall extensibility rapidly, requiring continuous auxin availability, ATP, active ATPases, protein synthesis, and turgor. To induce cell elongation, auxin must bind to receptors either inside the cell or at the plasma membrane, leading to wall loosening outside the cell. Protons, released into the apoplastic solution in response to auxin, play a crucial role in acidifying the wall and triggering the activity of wall-loosening enzymes. These enzymes, called wall loosening proteins (WLP), are believed to cleave load-bearing bonds in hemicelluloses, particularly xyloglucan in dicot walls, facilitating cell expansion. The exact identity of these bonds and enzymes remains a subject of ongoing research (Cleland 2004).

3.2 Function of auxin in tropisms

Research on the regulation of plant tropisms has always been closely related to efforts to uncover the processes behind polar auxin transport in higher plants. Charles Darwin proposed a theory as early as the nineteenth century, speculating that directed plant growth could be regulated by a transmissible signal in response to external stimuli. Auxin was discovered by plant physiologists to be a potential chemical that might mediate tropic growth responses much later. But it wasn't until the development of *Arabidopsis* genetics and cutting-edge molecular methods at the close of the 20th century that auxin-signalling pathways could be identified, leading to mechanistic understanding of the regulation of polar auxin transport and its relevance for plant tropisms (Retzer et al. 2014).

Adaptive development of plants to variations in light and gravity vectors necessitates an intricate signal transduction pathway. Auxin is associated with both gravitropism and phototropism, albeit many of the specifics of the mechanisms by which these divergent growth responses are

caused remain unclear. More specifically, it has been found and demonstrated that this mechanism requires the redistribution of auxin throughout tissues triggered by light or gravity. Auxin has been linked to tropisms through a variety of methods, such as the isolation of mutants with altered gravitropic or phototropic response resulting from changes in auxin transport or response, the identification of auxin gradients using radiolabelled auxin and auxin-inducible gene reporter systems, and the application of auxin transport inhibitors that prevent gravitropism and phototropism. Proteins that transport auxin have been identified and the mechanisms which determine auxin transport polarity have been explored. Finally, the data in support of several hypotheses for mechanisms by which auxin transport could be differentially regulated during gravitropism are examined (Muday 2001).

Auxin is essential in phototropic signal transduction, which involves the movement of auxin within plant tissues. The presence of indole-3-acetic acid (IAA), a form of auxin, is confirmed in various plant materials, including coleoptiles of oats and maize. PIN1, an auxin efflux carrier, is investigated for its cellular localization in the hypocotyl. Phototropic stimulation disrupts the basal localization of PIN1 more on the shaded side, and this response is absent in a phot1-deficient mutant, indicating the involvement of auxin transport in phototropism.

The NPH4 gene, encoding the auxin-regulated transcriptional activator ARF7, is linked to severe defects in both phototropism and gravitropism. Mutants of auxin-regulated genes, such as AUX/IAA19, also contribute to phototropism by influencing auxin-mediated growth asymmetry. These findings underscore the integral role of auxin in regulating

plant growth responses to light stimuli, emphasizing its significance in shaping plant development and behavior. Auxin is also implicated in the negative phototropism of plant roots and the phototropic curvature of *Phaseolus* leaf pulvinus. Phototropism, the response to light, in *Arabidopsis* primary roots involves signaling components NPH3 and RPT2, while in rice primary roots, CPT1 participates in this process. PIN3, a putative auxin efflux carrier, may also play a role in root phototropism. Additionally, the phototropic curvature of the *Phaseolus* leaf pulvinus is driven by turgor-dependent changes, where cells on the irradiated side shrink and those on the shaded side swell. This phenomenon, coupled with the observation that pulvinar protoplasts swell in response to applied auxin (IAA), suggests that lateral auxin redistribution might contribute to the turgor-driven phototropic curvature. Overall, auxin appears to be involved in the molecular and physiological processes underlying root and leaf phototropism.

3.3 Regulation of senescence and floral organ abscission by auxin

Transcription factors known as auxin response factors (ARFs) mediate reactions to the plant hormone auxin. Ellis at al. (2005) looked at Arabidopsis lines with T-DNA insertions in the ARF2 and AUXIN RESPONSE FACTOR1 (ARF1) genes. They discovered that ARF2 facilitates changes in Arabidopsis development from one stage to another. Arf2 mutant plants showed delays in a number of aging-related processes, such as floral organ abscission, rosette leaf senescence, silique ripening, and the start of flowering. Senescing leaves were shown to express ARF2. Independent of the ethylene and cytokinin response pathways, ARF2 controlled the senescence of leaves and the abscission of floral organs. ARF1 functions partially redundantly with ARF2, as evidenced by the enhancement

of numerous arf2 abnormalities by arf1 mutations. An arf1 mutation, on the other hand, enhanced transcription of Aux/IAA genes in Arabidopsis flowers, in contrast to arf2 mutations, validating earlier biochemical investigations (Fig. 1).

(I) (II)

Figure 1. (I) Natural senescence in wild-type and arf2 rosette leaves. Leaves and inflorescences from 8-week-old plants are laid out in order of emergence. (II) Effects of arf gene mutations on flower development. Stage 13 Columbia (A) and arf2-8 flowers (E). Primary inflorescences of Columbia (B), Ws-0 (C) arf1-5 arf2-8 (F) and arf1-4 dsARF2 (G) plants. Abscission zones of Columbia (D) and arf1-5 arf2-8 (H) floral organs. St, stamen; P, petal; Se, sepal. Arrowheads indicate the abscission zone (Ellis at al. 2005).

3.4 Auxin-mediated embryo patterning and control of cell division

The process of embryo growth in seed plants that starts with ovule fertilisation is known as embryogenesis. Following germination, this procedure will establish the general architecture of the newly formed plant. The final size and shape of the seed, which in turn affects the overall quality

and quantity of seed production, are determined by the coordinated growth of embryonic tissues (Han et al., 2019). Auxin allows proper polarity and patterning of the developing embryo through its distribution, which is maintained by a local auxin production together with well-coordinated transport. The auxin signalling pathway interprets these auxin maxima into proper developmental programs (Locascio et al. 2014; Schlereth et al. 2010).

Auxin plays a pivotal role in embryo patterning by influencing formative cell divisions and cell specification during embryo development. Coordinated cell divisions create distinct cell layers by orienting the division plane, and auxin disrupts the default geometric division rule, compelling cells to choose an alternative division wall. This control exerted by auxin is crucial for pattern formation in embryo morphogenesis. The influence of auxin on division plane orientation is achieved through its impact on the orientation of cortical microtubule arrays (CMAs), with a well-established link between CMA orientation and division plane orientation. Auxin-mediated stability of microtubules, along with factors such as cell shape and reduced edge catastrophe, governs CMA orientation, predicting division plane orientation and facilitating oriented cell division during early embryonic stages. Additionally, auxin's role extends to cell polarization relative to body axes, influencing division orientation during multicellular development. The SOSEKI proteins, particularly SOK1, are implicated in this process, with SOK1 expression being transcriptionally regulated by ARF5/MP. SOK1 is localized in specific regions of the embryo and its misexpression leads to altered cell division orientations across cell types. Despite these findings, the detailed cellular mechanisms underlying SOK1's activity in division plane orientation remain to be determined (Yoshida et al. 2014; Chakrabortty et al. 2018).

3.5 Contribution of auxin during seed germination

In *Arabidopsis thaliana*, abscisic acid (ABA) suppresses postgerminative growth and seed germination. Both auxin and jasmonic acid (JA) increase the activity of ABA. In a study conducted by Mei et al. (2023) demonstrate how exogenous auxin and JA complement one another to strengthen the ABA-induced delay in seed germination. For JA-promoted ABA responses, auxin production, perception, and signalling are essential. The auxin-dependent transcription factors ARF16 and AUXIN RESPONSE FACTOR10 (ARF10) interact with the JA signalling repressors known as JASMONATE ZIM-DOMAIN (JAZ). Combined ABA and JA treatment partially rescues the hyposensitive phenotype of JAZ-accumulating plants with impaired JA signalling, as ARF10 and ARF16 positively regulate JA-increased ABA responses. Moreover, ABI5, a crucial regulator of ABA signalling, is physically associated with ARF10 and ARF16. This means that ABI5 is primarily responsible for ARF16's capacity to elicit JA-mediated ABA responses. The transcriptional activity of ABI5 is stimulated by ARF10 and ARF16, while JAZ repressors counteract their effects. Altogether, the findings showed that auxin plays a role in the synergistic modulation of JA on ABA signalling and provide an explanation for how ARF10/16 collaborates with JAZ and ABI5 to integrate the pathways involved in auxin, JA, and ABA signalling in seed germination.

3.6 Initiation of apicobasal polarity: Emphasis on auxin

Auxin plays a crucial role in early embryonic patterning by influencing the initial separation of apical and basal cells after the first zygotic division. This separation establishes a foundation for subsequent patterning events, with fate decisions relying on the differential expression of

WUSCHEL-related homeobox (WOX) transcription factors along the apicobasal axis. Specifically, WOX2, WOX8/STPL, and WOX9 are expressed in different regions of the eight-celled embryo. Auxin response mediated by ARF5/MP is essential for these expression dynamics, as observed in arf5/mp and iaa12/bdl mutants, where the shift in WOX9 expression is compromised. WOX2 emerges as a key regulator in the WOX gene cascade, orchestrating embryonic shoot patterning. The interplay between WOX genes and auxin is evident in wox8 wox9 double mutants, where reduced PIN1 expression suggests the necessity of WOX8/WOX9 for normal PIN1 activity and localized auxin response maxima. Moreover, a genetic interaction between WOX2, WOX8, and ARF5/MP underscores their collective role in regulating PIN1 expression during cotyledon development, emphasizing the intricate connection between WOX genes, auxin-mediated responses, and potentially other auxin-independent factors in embryo patterning (Haecker et al. 2004; Breuninger et al. 2008).

3.7 Role of auxin in initiating SAM

In the process of initiating the Shoot Apical Meristem (SAM), auxin, the plant hormone, plays a crucial role. Initially, stem cells are specified between presumptive cotyledons in the globular embryo. During apical patterning, a shift from radial to bilateral symmetry occurs with the development of cotyledon primordia. The proper separation of cotyledon primordia is essential for SAM establishment (Lie et al. 2012).

SAM development and cotyledon separation are controlled by a network of genes, including CUP-SHAPED COTYLEDON1 (CUC1), CUC2, CUC3, and SHOOT MERISTEMLESS (STM). Three key factors in the auxin transport and response pathway—PIN1, PINOID (PID), and

ARF5/MP—regulate the expression of CUC1 and CUC2, contributing to cotyledon separation and bilateral symmetry. PIN1 and PID work together to promote auxin accumulation in cotyledon primordia, inhibiting the expression of CUC1 and CUC2. This process, facilitated by auxin, is crucial for the establishment of bilateral symmetry (Vroemen et al. 2003).

Furthermore, the WOX2 module, which includes WOX2, WOX8, WOX1, WOX3, and WOX5, is essential for the initiation of the embryonic apical meristem. This module promotes the development of a three-layered shoot meristem by regulating cell division patterns, including the presumptive SAM region. By promoting PIN1 expression, the WOX2 module prevents auxin accumulation in the SAM region. Additionally, the WOX2 module enhances the expression of HD-ZIP III genes, known regulators of embryonic apical fate, suggesting that HD-ZIP III TFs act downstream of the WOX2 module in the same genetic pathway to regulate stem cell initiation during embryonic apical patterning. In summary, auxin, through the PIN1-mediated pathway, helps establish the bilateral symmetry necessary for SAM formation, and the WOX2 module, in coordination with auxin and cytokinin pathways, contributes to stem cell initiation and protects them from differentiation, ensuring correct specification of the SAM region in the apical domain (Zhang et al. 2017).

3.8 Role of Auxin in Embryonic Root Formation

In embryonic root formation, auxin has a significant role in initiating the Root Apical Meristem (RAM). The asymmetric division of the hypophysis, marked by the initiation of RAM at the globular stage, is essential. The specification of the hypophysis and subsequent RAM development are dependent on auxin signaling. Mutants with disrupted auxin

signaling, such as MP and BDL, exhibit a rootless phenotype. Auxin transporters, including AUX1, LAX2, and PIN1, are targets of the MP/BDL signaling pathway, guiding an auxin flow toward the hypophysis (Capron et al. 2009).

MP, a transcription factor, directly targets genes such as TMO5 and TMO7, crucial for RAM specification. TMO7 acts as an intercellular signal in the hypophysis for RAM initiation. Additionally, NTT, WIP4, and WIP5, also direct targets of MP, promote the asymmetric division marking RAM initiation (Weijers et al. 2006). The PLT genes, including PLT1 and PLT2, form an indispensable gene network regulating RAM establishment. PLT genes act downstream of auxin response, and their expression depends on MP/ARF5 and NPH4/ARF7. PIN-mediated auxin accumulation in the basal domain is crucial for initiating and establishing RAM by transcriptionally regulating PLT genes (Crawford et al. 2015).

Furthermore, PLT genes are implicated in vascular regeneration, modulating auxin biosynthesis and directly regulating CUC2 expression. This intricate mechanism, observed in leaves, may also exist in the embryo, highlighting the multifaceted role of auxin in embryonic root formation (Radhakrishnan et al. 2020).

4. Signal transduction of auxin

Clarifying the molecular pathways behind hormone function is one of the numerous difficulties facing plant hormone research. Auxin signal transduction pathway's present working model is primarily derived from hormone response pathways that have been reported in other systems. It is believed that auxin is recognised and bound by receptors within cells as well

as on the cell surface with a high affinity. Following receptor binding, a sequence of biochemical and molecular processes would take place, eventually resulting in physiological growth responses that are visible, like cell elongation, division, and/or differentiation. Because of the identification of several crucial regulatory elements of the pathway, we now have a far better knowledge of the molecular mechanisms governing auxin action than we had ten years ago (Hagen et al. 2004).

Auxin can cause both specialised and general transcriptional reactions when it is perceived in the nucleus. Auxin/INDOLE-3-ACETIC ACID (Aux/IAA) transcriptional repressors, AUXIN RESPONSE FACTOR (ARF) transcription factors, and F-box TRANSPORT INHIBITOR RESPONSE 1/AUXIN SIGNALLING F-BOX PROTEIN (TIR1/AFB) auxin co-receptors are the three protein families that comprise the essential elements of the auxin signalling machinery. Auxin stimulates TIR1/AFB and Aux/IAA protein interaction, which leads to ARF repression being released and the Aux/IAAs being degraded (Salehin et al. 2015).

Auxin regulates gene expression through a complex pathway involving transcriptional responses mediated by Auxin Response Factors (ARFs) and Aux/IAA transcriptional repressors. At low auxin levels, Aux/IAAs inhibit ARF activity. However, in the presence of auxin, TIR1/AFB proteins bind to Aux/IAAs, leading to their degradation. This process triggers rapid induction of auxin-responsive genes, forming negative-feedback loops. Aux/IAAs have three functional domains: a leucine repeat EAR motif for repression, an internal domain II with a degron motif for interaction with TIR1/AFBs, and a C-terminal PB1 domain for interactions and self-dimerization. The degron motif determines Aux/IAA

stability, and other sequences outside domain II contribute to TIR1/AFB binding and degradation. This intricate mechanism highlights auxin's role in finely regulating gene expression for plant growth and development (Wang et al. 2016).

Auxin perception initiates ARF-mediated transcriptional responses after the degradation of Aux/IAA transcriptional repressors. ARF factors bind to auxin-responsive gene promoters through cis-regulatory AuxREs, often containing the core element TGTC. Structural analyses reveal specific motifs (e.g., TGTCCG) preferred by different ARFs. ARFs, characterized as transcriptional activators or repressors, possess an N-terminal DNA-binding domain, a variable middle region, and a C-terminal PB1 dimerization domain. ARFs can form dimers, influencing transcriptional responses, and their interactions with Aux/IAAs are vital for efficient ARF repression. Structural studies uncover a PB1 domain facilitating various dimerizations and oligomerizations, offering insights into the intricate regulation of auxin-responsive gene expression (Wang and Estelle 2014).

Auxin-mediated gene repression involves ARFs recruiting Aux/IAAs to target gene promoters, inducing chromatin modifications that decrease gene accessibility. Aux/IAAs interact with corepressor proteins TPL and TPR, engaging histone deacetylases for transcriptional repression. A novel auxin-mediated chromatin switch was revealed, where ARF5 interacts with BRM and SYD, subunits of the SWI/SNF complex, promoting gene activation in flower primordia. This interaction enhances DNA accessibility to transcription factors, facilitating target gene induction. Aux/IAAs prevent BRM and SYD association with gene promoters, allowing the dynamic

switch between gene repression and activation orchestrated by auxin and Aux/IAA degradation (Kagale and Rozwadowski 2011; Wu et al. 2015).

5. Conclusion

Plant hormone auxin is engaged in an incredibly wide range of biological processes. These span from fundamental biological functions like endocytosis, cell polarity, and cell cycle regulation to specialized reactions like cell elongation and differential growth, as well as large-scale occurrences like embryogenesis, tissue patterning, and organ de novo development. Despite the fact that auxin study dates back more than a century, a thorough understanding of how auxin controls such a broad spectrum of responses remains a long way off. The auxin molecule itself may hold some of the answers to this query. There are compounds that resemble auxins that occur naturally, and these molecules may be involved in particular cellular and developmental processes. Moreover, auxin is intracellularly compartmentalised, actively changed, and metabolised, all of which can significantly affect its availability and action.

References

Abel S, Theologis A (2010) Odyssey of auxin. Cold Spring Harbor Perspectives in Biology 2(10): 4572.

Breuninger H, Rikirsch E, Hermann M, Ueda M, Laux T (2008) Differential expression of WOX genes mediates apical-basal axis formation in the *Arabidopsis* embryo. Dev Cell 14: 867–876.

Brumos J, Alonso JM, Stepanova AN (2014) Genetic aspects of auxin biosynthesis and its regulation. Physiol Plant 151(1): 3–12.

Capron A, Chatfield S, Provart N, Berleth T (2009) Embryogenesis: pattern formation from a single cell. *Arabidopsis* B 7: 126.

Casanova E, Trillas MI, Moysset L, Vainstein A (2005) Influence of rol genes in floriculture. Biotechnol Adv 23(1): 3–39.

Chakrabortty B, Willemsen V, de Zeeuw T, Liao CY, Weijers D, Mulder B, Scheres B (2018) A plausible microtubule- based mechanism for cell division orientation in plant embryogenesis. Curr Biol 28: 3031–3043.

Chandler JW (2009) Auxin as compere in plant hormone crosstalk. Planta 231(1): 1–12.

Cleland RE (2004) Auxin and cell elongation. In: Davies P J (ed) Plant hormones: biosynthesis, signal transduction, action. Kluwer Academic Publishers, Dordrecht, pp 223–229.

Crawford BCW, Sewell J, Golembeski G, Roshan C, Long JA, Yanofsky MF (2015) Genetic control of distal stem cell fate within root and embryonic meristems. Science 347: 655– 659.

Dai X, Mashiguchi K, Chen Q, Kasahara H, Kamiya Y, Ojha S, DuBois J, Ballou D, Zhao Y (2013) The biochemical mechanism of auxin biosynthesis by an arabidopsis YUCCA flavin-containing monooxygenase. J Biol Chem 288(3): 1448–1457.

Darwin C, Darwin F (1880) The power of movement in plants. John Murray, London, pp 87–92.

Di DW, Zhang C, Luo P, An CW, Guo GQ (2016) The biosynthesis of auxin: how many paths truly lead to IAA? Plant Growth Regul 78: 275–285.

Ellis C M, Nagpal P, Young J C, Hagen G, Guilfoyle T J, Reed J W (2005) AUXIN RESPONSE FACTOR1 and AUXIN RESPONSE FACTOR2 regulate senescence and floral organ abscission in *Arabidopsis thaliana*. Development 132(20): 4563–4574.

Epstein E, Cohen J, Slovin J (2002) The biosynthetic pathway for indole-3-acetic acid changes during tomato fruit development. Plant Growth Regul 38: 15–20.

Haecker A, Groß-Hardt R, Geiges B, Sarkar A, Breuninger H, Herrmann M, Laux T (2004) Expression dynamics of WOX genes mark cell fate decisions during early embryonic patterning in Arabidopsis thaliana. Development 131: 657–668.

Hagen G, Guilfoyle T J, Gray W M (2004) Auxin signal transduction. In: Davies P J (ed) Plant hormones: biosynthesis, signal transduction, action. Kluwer Academic Publishers, Dordrecht, pp 295–301.

Han Q, Bartels A, Cheng X, Meyer A, An YC, Hsieh TF, Xiao W (2019) Epigenetics regulates reproductive development in plants. Plants (Basel) 8: 564.

Kagale, S and Rozwadowski K (2011) EAR motif-mediated transcriptional repression in plants: an underlying mechanism for epigenetic regulation of gene expression. Epigenetics 6: 141–146.

Last RL, Bissinger PH, Mahoney DJ, Radwanski ER, Fink GR (1991) Tryptophan mutants in Arabidopsis: the consequences of dupli- cated tryptophan synthase beta genes. Plant Cell 3(4): 345–358.

Lehmann T, Hoffmann M, Hentrich M, Pollmann S (2010) Indole-3-acetamide-dependent auxin biosynthesis: a widely distributed way of indole-3-acetic acid production? Eur J Cell Biol 89(12): 895–905.

Lie C, Kelsom C, Wu X (2012) WOX2 and STIMPY-LIKE/ WOX8 promote cotyledon boundary formation in *Arabidopsis*. Plant Journal 72: 674–682.

Locascio A, Roig-Villanova I, Bernardi J, Varotto S (2014) Current perspectives on the hormonal control of seed development in *Arabidopsis* and maize: a focus on auxin. Front Plant Sci 5: 412.

Mano Y, Nemoto K (2012) The pathway of auxin biosynthesis in plants. J Exp Bot 63(8): 2853–2872

Mei S, Zhang M, Ye J, Du J, Jiang Y, Hu Y (2023) Auxin contributes to jasmonate-mediated regulation of abscisic acid signaling during seed germination in *Arabidopsis*. The Plant Cell 35(3): 1110–1133

Muday GK (2001) Auxins and tropisms. J Plant Growth Regul 20(3): 158.

Nafisi M, Goregaoker S, Botanga CJ, Glawischnig E, Olsen CE, Halkier BA, Glazebrook J (2007) Arabidopsis cytochrome P450 monooxygenase 71A13 catalyzes the conversion of indole-3- acetaldoxime in camalexin synthesis. Plant Cell 19(6): 2039–2052.

Pérez-Llorca M, Muñoz P, Müller M, Munné-Bosch S (2019) Biosynthesis, metabolism and function of auxin, salicylic acid and melatonin in climacteric and non-climacteric fruits. Front Plant Sci 10: 136.

Pollmann S, Neu D, Weiler EW (2003) Molecular cloning and characterization of an amidase from *Arabidopsis thaliana* capable of converting indole-3-acetamide into the plant growth hormone, indole-3-acetic acid. Phytochemistry 62(3): 293–300.

Quittenden LJ, Davies NW, Smith JA, Molesworth PP, Tivendale ND, Ross JJ (2009) Auxin biosynthesis in pea: characterization of the tryptamine pathway. Plant Physiol 151(3): 1130–1138.

Radwanski ER, Last RL (1995) Tryptophan biosynthesis and metabolism: biochemical and molecular genetics. Plant Cell 7(7): 921–934.

Retzer K, Korbei B, Luschnig C (2014) Auxin and tropisms. Auxin and its role in plant development 43: 361–387.

Reverberi M, Fanelli C, Zjalic S, Briganti S, Picardo M, Ricelli A, Fabbri AA (2005) Relationship among lipoperoxides, jasmonates and indole-3-acetic acid formation in potato tuber after wounding. Free Radic Res 39(6): 637–647.

Ribnicky DM, Cohen JD, Hu WS, Cooke TJ (2002) An auxin surge following fertilization in carrots: a mechanism for regulating plant totipotency. Planta 214(4): 505–509.

Salehin M, Bagchi, Estelle M (2015) SCFTIR1/AFB-based auxin perception: mechanism and role in plant growth and development. Plant Cell 27: 9–19.

Sauer M, Robert S, Kleine-Vehn J (2013) Auxin: simply complicated. J Exp Bot 64(9): 2565–2577.

Schlereth A, Möller B, Liu W, Kientz M, Flipse J, Rademacher EH, Schmid M, Jürgens G, Weijers D (2010) MO- NOPTEROS controls embryonic root initiation by regulating a mobile transcription factor. Nature 464: 913–916.

Simon S, Petrasek J (2011) Why plants need more than one type of auxin. Plant Sci 180(3): 454–460.

Stepanova AN, Robertson-Hoyt J, Yun J, Benavente LM, Xie DY, Dolezal K, Schlereth A, Jurgens G, Alonso JM (2008) TAA1- mediated auxin biosynthesis is essential for hormone crosstalk and plant development. Cell 133(1): 177–191.

Sugawara S, Hishiyama S, Jikumaru Y, Hanada A, Nishimura T, Koshiba T, Zhao Y, Kamiya Y, Kasahara H (2009) Biochemical analyses of indole-3-acetaldoxime-dependent auxin biosynthesis in *Arabidopsis*. Proc Natl Acad Sci USA 106(13): 5430–5435.

Tivendale ND, Ross JJ, Cohen JD (2014) The shifting paradigms of auxin biosynthesis. Trends Plant Sci 19(1): 44–51.

Varapparambathu V, Toms A, Kerstens M, Valsakumar D, Landge AN, Shaji A (2020) A coherent feed-for- ward loop drives vascular regeneration in damaged aerial organs of plants growing in a normal developmental context. Development 147: 23.

Vorwerk S, Biernacki S, Hillebrand H, Janzik I, Muller A, Weiler EW, Piotrowski M (2001) Enzymatic characterization of the recombinant *Arabidopsis thaliana* nitrilase subfamily encoded by the NIT2/NIT1/NIT3-gene cluster. Planta 212(4): 508–516.

Vroemen CW, Mordhorst AP, Albrecht C, Kwaaitaal MACJ, de Vries SC (2003) The CUP-SHAPED COTYLEDON3 gene is required for boundary and shoot meristem formation in *Arabidopsis*. Plant Cell 15: 1563–1577.

Wang B, Chu J, Yu T, Xu Q, Sun X, Yuan J, Xiong G, Wang G, Wang Y, Li J (2015) Tryptophan-independent auxin biosynthesis contributes to early embryogenesis in *Arabidopsis*. Proc Natl Acad Sci USA 112(15): 4821–4826.

Wang R and Estelle M (2014) Diversity and specificity: auxin perception and signaling through the TIR1/AFB pathway. Curr Opin Plant Biol 21: 51–58.

Wang R, Zhang Y, Kieffer M, Yu H, Kepinski S and Estelle M (2016) HSP90 regulates temperature-dependent seedling growth in Arabidopsis by stabilizing the auxin co-receptor F-box protein TIR1. Nat Commun 7: 10269.

Weijers D, Schlereth A, Ehrismann JS, Schwank G, Kientz M, Jürgens G (2006) Auxin triggers transient local signaling for cell specification in *Arabidopsis* embryogenesis. Dev Cell 10: 265–270.

Went FW, Thimann KV (1937) Phytohormones. Macmillan, New York, pp 123–128.

Wildman SG (1997) The auxin-A, B enigma: Scientific fraud or scientific ineptitude? Plant Growth Regul 22: 37–68.

Woodward AW, Bartel B (2005) Auxin: regulation, action, and interaction. Ann Bot 95(5): 707–735.

Wu M F, Yamaguchi N, Xiao J, Bargmann B, Estelle M, Sang Y and Wagner D (2015) Auxin-regulated chromatin switch directs acquisition of flower primordium founder fate. eLife 4: 9269–9289.

Wu M, Wu J, Gan Y (2020) The new insight of auxin functions: transition from seed dormancy to germination and floral opening in plants. Plant Growth Regul 91: 169–174.

Yang ZB, Geng X, He C, Zhang F, Wang R, Horst WJ, Ding Z (2014) TAA1-regulated local auxin biosynthesis in the root-apex transition zone mediates the aluminum-induced inhibition of root growth in *Arabidopsis*. Plant Cell 26(7): 2889–2904.

Yoshida S, de Reuille PB, Lane B, Bassel GW, Prusinkiewicz P, Smith RS, Weijers D (2014) Genetic control of plant development by overriding a geometric division rule. Dev Cell 29: 75–87.

Zhang Z, Tucker E, Hermann M, Laux T (2017) A molecular framework for the embryonic initiation of shoot meristem stem cells. Dev Cell 40: 264–277.

Zhao Y (2014) Auxin biosynthesis. *Arabidopsis* B 12: e0173.

Zhao Y, Christensen SK, Fankhauser C, Cashman JR, Cohen JD, Weigel D, Chory J (2001) A role for flavin monooxygenase-like enzymes in auxin biosynthesis. Science 291(5502): 306–309.

Cytokinin Complexity: Insights into Plant Growth Regulation

Shifa M.

PG Department of Botany, Korambayil Ahamed Haji Memorial Unity Women's College
Manjeri, Malappuram, 676122, Kerala, India.
Email: shifashibu7@gmail.com

Abstract

This book chapter provides a comprehensive examination of the versatile roles played by cytokinin, a crucial plant hormone, in shaping various facets of plant growth and development. Focusing on its impact on root and shoot architecture, female gametophyte development, vascular tissue differentiation, and root nodule formation, the chapter elucidates the intricate molecular mechanisms and signaling pathways through which cytokinin orchestrates these intricate processes. The interplay between cytokinins and other plant hormones, such as auxins, helps orchestrate developmental processes like cell division, shoot and root formation, and vascular differentiation. By unraveling the molecular cross-talk between cytokinin and key developmental events, this chapter aims to offer a deeper understanding of the hormone's regulatory functions, providing valuable insights for researchers, educators, and students engaged in unraveling the complexities of plant hormone signaling networks. The exploration of cytokinin's diverse functions underscores its central role in coordinating plant growth and development at multiple levels.

Keywords: Biosynthesis, Cytokinin, Developmental mechanism, Plant hormone, Signaling pathways, Tranz-zeatin.

1. Introduction

Cytokinin, recognized as a fundamental plant growth phytohormone, plays a pivotal role in fostering both cell division and cell differentiation within plants. These adenine derivatives feature a variable side chain at the N6 position of the purine (Sakakibara 2006). Operating as a key growth-promoting phytohormone, cytokinin exerts its influence across various facets of plant growth and development (Fahad et al. 2015). The signaling pathway of cytokinin is facilitated by a two-component system that orchestrates the sequential transfer of phosphoryl groups from receptors to downstream effectors. In Arabidopsis, upon cytokinin binding, receptors belonging to a small class of His kinases undergo autophosphorylation at a highly conserved histidine residue. Subsequently, the phosphoryl group is translocated to PHOSPHOTRANSFER PROTEINS and further to RESPONSE REGULATORS. This cascade ultimately activates the transcription of downstream effector genes, thereby initiating the signaling pathway. This chapter primarily consolidates recent advancements and our present comprehension of cytokinin metabolism, translocation, signal transduction, regulatory mechanisms, and the diverse physiological roles cytokinin plays in the intricate tapestry of plant growth and development (Kakimoto 2001).

2. Discovery

Cytokinins, classified as phytohormones, play a crucial role in promoting cell division and cell differentiation in plants (Haberer and Kieber 2002). These adenine derivatives carry either an isoprene-derived or an aromatic side chain at the N6 position of the purine (Sakakibara 2006). The discovery of cytokinins can be traced back to the 1950s when Folke Skoog

and colleagues isolated the first cytokinesis-promoting factor, kinetin, from autoclaved herring sperm DNA (Feng et al. 2017). Subsequently, other growth-promoting factors similar to kinetin were identified in various plants. The first naturally occurring cytokinin, trans-zeatin (tZ), was isolated from maize endosperm. Over the years, researchers have identified compounds with cytokinin activities from numerous plant species, with isopentenyladenine (iP) and tZ being the most prevalent and extensively studied natural cytokinins. The discovery of cytokinins not only advanced plant tissue culture methods but also significantly influenced studies in plant biology. Cytokinins are involved in regulating diverse processes in plant growth and development, encompassing aspects such as female gamete and embryo development, seed germination, vascular development, shoot apical meristem development, photomorphogenesis, leaf senescence, and floral development (Chen and Leisner 1984)). Additionally, cytokinins play a crucial role in regulating adaptive responses to environmental stresses (Bielach et al. 2017). In recent decades, the identification of enzymes controlling the modification and activity of cytokinins has provided valuable insights into the fundamental molecular mechanisms of cytokinin biosynthesis (Shakakibara 2006).

3. Structure and types of cytokinin

Cytokinins, as adenine derivatives, exhibit structural diversity based on the configuration of the N6-side chain, leading to the classification of isoprenoid cytokinins and aromatic cytokinins. Isoprenoid cytokinins, such as N6-(Δ2-isopentenyl)-adenine (iP) and zeatin, feature an isopentenyl side chain, while aromatic cytokinins, like kinetin, N6-benzyl adenine (6-BA), and topolin, possess a benzyl or hydroxybenzyl group at the N6 position. The trans- and cis-configurations of the hydroxylated side chain result in trans-

zeatin (tZ) and cis-zeatin (cZ), respectively. Aromatic cytokinins, though present in lower quantities, include ortho-topolin (oT), meta-topolin (mT), and methoxy derivatives of 6-BA (Feng et al. 2017). These cytokinins often occur as nucleobases or in conjugation with various moieties, such as nucleosides, nucleotides, and glycosides. The nucleobases typically serve as the active form, while the nucleosides act as reservoirs for cytokinin storage.

Beyond naturally occurring cytokinins, synthetic derivatives, including phenylurea-type, thidiazuron, and adenine-type kinetin, have been generated. The biological activities of cytokinins vary across plant species, tissues, and developmental stages, with different cytokinins exhibiting distinct affinities for receptors. For instance, cytokinin receptors generally show high affinity for free bases and low affinity for riboside derivatives. Specific cytokinin receptors in plants, such as Arabidopsis AHK3 and maize ZmHK1 and ZmHK2, display preferences for particular ligands, underscoring the specificity of cytokinin signaling in diverse plant systems (Kieber and Schaller 2018).

4. Cytokinin biosynthesis

Cytokinins, integral to plant growth regulation, undergo intricate biosynthetic processes involving the de novo synthesis and tRNA degradation pathways, contributing to the diversity of cytokinin types in plants (Frebort 2011). In the de novo synthesis pathway, the rate-limiting step is the transfer of the prenyl moiety from dimethylallyl diphosphate (DMAPP) to the N6 position of ATP, ADP, or AMP. This pivotal reaction is catalyzed by adenylate isopentenyltransferase (IPT), and in Arabidopsis, the genome contains nine IPT genes, including AtIPT1 to AtIPT9. Notably, AtIPT4 and AtIPT8 (PGA22) overexpression results in increased iP levels

and a typical cytokinin response, highlighting their significant roles. Furthermore, the preference of Arabidopsis IPTs for ADP or ATP leads to the production of iP ribosides, mainly iPRDP and iPRTP. Subsequent hydrolytic reactions release iP riboside (iPR) and free base iP.

The tRNA degradation pathway involves tRNA isopentenyl transferase (tRNA-IPT) enzymes, such as AtIPT2 and AtIPT9 in Arabidopsis, which transfer an isopentenyl unit from DMAPP to the N6 position of the nucleotide adjacent to the 3′-end of the tRNA anticodon. A loss-of-function mutant of both AtIPT2 and AtIPT9 results in a significant reduction in cZR and cZRMP contents, emphasizing the crucial role of tRNA degradation as the primary source for cZ-type cytokinins (Feng et al. 2017). Biochemical studies have revealed two possible pathways for trans-zeatin (tZ) biosynthesis: an iPRMP-dependent pathway, catalyzed by cytochrome P450 monooxygenase CYP735A1 and CYP735A2, and an iPRMP-independent pathway, proposing zeatin synthesis from AMP and side chain precursor by IPT (Sakakibara 2006). The complex biosynthetic pathways of cytokinins involve multiple enzymes and precursor molecules, resulting in the production of diverse cytokinin types. These pathways play pivotal roles in regulating various aspects of plant growth and development, illustrating the intricate interplay of cytokinins in the dynamic physiology of plants (Fig. 1) (Sakakibara 2006).

4.1 Activation of cytokinins

The two proposed pathways for cytokinin activation, namely the one-step and two-step reaction pathways, provide insights into the dynamic processes governing the conversion of inactive riboside forms to active cytokinins in plants. The two-step pathway, considered the predominant

route, involves sequential cleavage of the phosphate and sugar moieties from nucleotides, ultimately yielding the active nucleobases iP and tZ. This pathway has been extensively studied, with the identification of key enzymes such as nucleoside N-ribohydrolase (NRH) responsible for the second hydrolytic step. In contrast, the one-step pathway, exemplified by the action of LONELY GUY (LOG) proteins, facilitates direct conversion of cytokinin nucleotides to their free-base forms, bypassing the intermediate nucleoside stage. The identification of LOG homologs in various plant species, including the nine LOG genes in Arabidopsis, suggests a conserved mechanism across monocots and dicots. The coexistence of these two pathways highlights the intricacies of cytokinin activation, reflecting the adaptability of plants to different environmental cues and growth conditions.

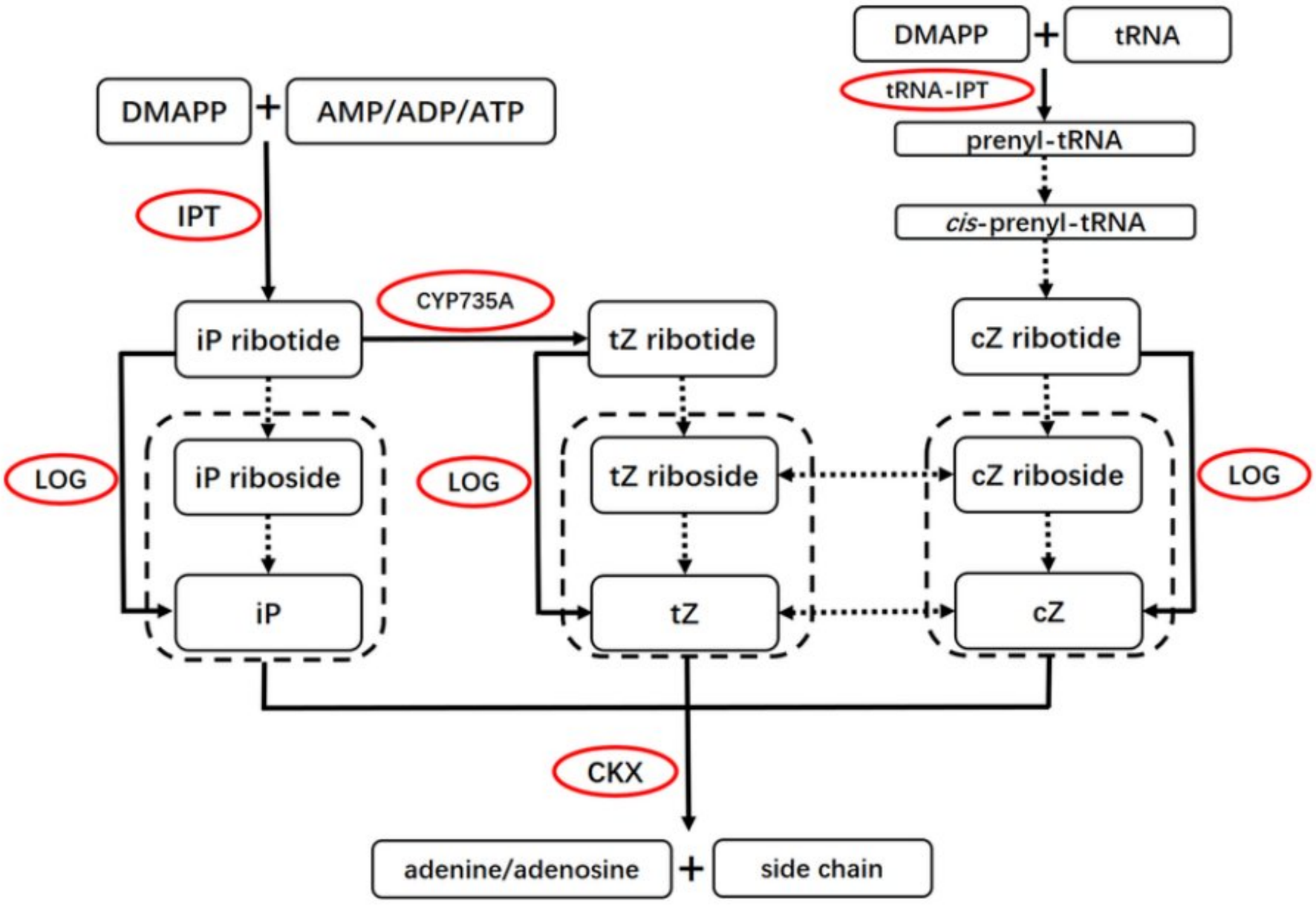

Figure 1. Basic scheme for the cytokinin biosynthesis and degradation pathways. Solid arrows indicate pathways with genes that are known, and

dotted arrows indicate pathways with genes that remain to be identified. The enzymes are marked by red frames. The iP, Z and their ribosides inside the dotted boxes could be degraded by CKX. cZ, cis-zeatin; DMAPP, dimethylallyldiphosphate; CKX, cytokinin oxidase/dehydrogenase; cZ, cis-zeatin; DMAPP, dimethylallyl diphosphate; iP, N 6-(Á 2-isopentenyl) adenine; IPT, adenosine phosphate-isopentenyltransferase; LOG, LONELY GUY; tRNA-IPT, tRNAisopentenyltransferase; tZ, trans-zeatin; Z, zeatin. This figure was modified and redrawn from reference (Kudo et al. 2010).

4.2 Sites of cytokinin biosynthesis

Earlier studies proposed that cytokinin biosynthesis primarily takes place in roots; however, recent investigations indicate a more widespread distribution throughout the entire plant. AtIPT genes, key players in cytokinin biosynthesis, exhibit expression in various shoot tissues, including leaves, stems, flowers, and siliques. The specific AtIPT gene expression patterns further highlight the spatial differentiation in cytokinin biosynthesis. For instance, AtIPT1 is predominantly expressed in the vascular stele of roots, leaf axils, ovules, and immature seeds, while AtIPT3 is observed in the phloem companion cells. AtIPT4 and AtIPT8 show expression in immature seeds, particularly in the chalazal endosperm, and AtIPT5 is present in lateral root primordia, root-cap columella, upper parts of young inflorescences, and fruit abscission zones. AtIPT7 exhibits expression in the endodermis of the root elongation zone, trichomes on young leaves, and pollen tubes, indicating diverse sites of cytokinin biosynthesis within the plant. Additionally, the differential expression of LOG genes, which play a role in cytokinin activation, across various tissues during plant development underscores the varied distribution of cytokinin types within different plant tissues (Takei et al. 2001).

Interestingly, the distribution of cytokinins is not uniform across plant tissues. Xylem sap is characterized by predominantly containing trans-zeatin (tZ)-type ribosides, while phloem sap is enriched in isopentenyladenine (iP) and cis-zeatin (cZ)-type ribosides. The dominance of tZ-type cytokinins in xylem sap aligns with the expression patterns of CYP735A genes, responsible for the hydroxylation of the isopentenyl side chain, primarily occurring in roots. This spatial differentiation in cytokinin distribution and biosynthetic gene expression suggests a finely tuned regulatory mechanism that adapts to the distinct physiological needs of different plant tissues (Choudhary 2013).

4.3 Regulation of cytokinin biosynthesis

The synthesis of cytokinins is intricately regulated by a variety of factors, encompassing hormonal signals and macronutrient availability. In Arabidopsis, cytokinins play a crucial role in promoting cell division by counteracting the effects of auxin. Specifically, auxin induces the expression of AtIPT5 and AtIPT7, while cytokinins exert a repressive influence on AtIPT1, AtIPT3, AtIPT5, and AtIPT7 in the shoot meristem. This dynamic interplay between cytokinins and auxin highlights their antagonistic relationship in regulating cellular processes. Additionally, auxin negatively regulates cytokinin biosynthesis in the nodal stem of pea plants by suppressing the expression of the PsIPT gene (Muller 2011).

Furthermore, macronutrients contribute to the intricate regulation of cytokinin biosynthesis. Nitrate, a key macronutrient, has been shown to enhance the accumulation of various cytokinins in maize and trans-zeatin (tZ)-type cytokinins in Arabidopsis roots. This effect is attributed to the induction of AtIPT3 expression, as evidenced by a significant reduction in

nitrate-dependent cytokinin accumulation in an ipt3 null mutant. Other macronutrients, including sulfate and phosphate, also play regulatory roles in modulating AtIPT3 expression.

Beyond transcriptional regulation, posttranslational modifications also play a pivotal role in fine-tuning cytokinin biosynthesis. For instance, AtIPT3 contains characteristic CaaX boxes—short cysteine-containing motifs recognized by farnesyl transferase. Farnesylation directs the subcellular localization of AtIPT3, with nonfarnesylated AtIPT3 primarily localized in plastids, while farnesylated AtIPT3 is found in the nucleus and cytoplasm. This dual-layered regulation, involving both transcriptional and posttranslational mechanisms, highlights the sophisticated control mechanisms that govern cytokinin biosynthesis in response to various environmental and developmental cues (Ahmad et al. 2023).

4.4 Cytokinin degradation

In addition to cytokinin biosynthesis and activation, the degradation of cytokinins is a crucial regulatory mechanism that helps maintain optimal cytokinin levels in plants. Cytokinin oxidase/dehydrogenase (CKX) enzymes, classified as oxidoreductases with a flavin adenine dinucleotide (FAD)-binding motif, play a central role in this process. These enzymes irreversibly degrade endogenous cytokinins with an unsaturated side chain, including isopentenyladenine (iP), cis-zeatin (cZ), and trans-zeatin (tZ) (Feng et al. 2017).

Maize, for instance, possesses a family of 13 ZmCKX genes expressed in various tissues such as grains, roots, leaves, and immature ears. Among these, ZmCKX1 and ZmCKX3 have been demonstrated to exhibit

enzymatic activity (Vyroubalova et al. 2009). Similarly, Arabidopsis harbors seven CKX genes (AtCKX1 to AtCKX7), each displaying distinct expression patterns across different tissues. AtCKX1 is primarily expressed in the shoot apex, young floral tissues, and the root-hypocotyl junction, while AtCKX2 is found in the shoot apex and stipules. AtCKX4 is predominantly expressed in trichomes, stipules, and root caps, and AtCKX5 expression is confined to the edges of the youngest emerging leaves and in shoot and root meristems. AtCKX6, on the other hand, is expressed in the vasculature.

The subcellular localization of CKX enzymes further contributes to their functional specificity. AtCKX1 and AtCKX3 are found in the plant vacuole, while AtCKX2 localizes in the endoplasmic reticulum. Altering the expression of CKX genes results in significant phenotypic changes in various plant species. Overexpression of AtCKX1 to AtCKX4 in Arabidopsis, driven by the 35S promoter, leads to reduced cytokinin levels and cytokinin-deficient phenotypes in shoots and roots. Similarly, overexpressing the orchid DSCKX1 gene in Arabidopsis results in developmental phenotypes due to decreased cytokinin levels (Wang et al. 2020).

Interestingly, manipulating CKX gene expression can have profound effects on crop yield. For instance, in rice, the OsCKX2 gene, encoding a cytokinin oxidase, is linked to a quantitative trait locus (GN1A) controlling grain yield. Transgenic rice plants expressing antisense constructs of OsCKX2 show elevated cytokinin levels, leading to increased inflorescence meristems and reproductive organs, ultimately enhancing grain productivity. In other cases, such as with AtCKX3 and AtCKX5 in Arabidopsis, root-specific overexpression of CKX genes promotes root growth, contributing to

improved drought tolerance and nutrient absorption.These findings underscore the significance of CKX enzymes as pivotal targets for molecular strategies aimed at enhancing crop yields through the precise modulation of cytokinin levels (Chen et al. 2022).

4.5 Cytokinin transport

The transport of different types of cytokinins in plants has been revealed through studies employing reciprocal grafting with ipt1, 3, 5, 7 mutants and wild-type Arabidopsis plants. These investigations indicate that isopentenyladenine (iP) is predominantly transported from shoots to roots, while trans-zeatin (tZ) might primarily move from roots to shoots. The importance of root-to-shoot transport of tZ for shoot development has been underscored by analyzing the phenotype of the cyp735a1 cyp735a2 double mutant. This highlights the necessity of an efficient transport system for cytokinins to reach their target cells. Three main types of proteins contribute to cytokinin transport: purine permeases (PUPs), nucleoside transporters (equilibrative nucleoside transporters, ENTs), and ATP-binding cassette (ABC) transporters.

Arabidopsis has two identified PUP genes, AtPUP1 and AtPUP2, which encode transporters localized to the plasma membrane, facilitating cytokinin uptake into cells. These PUPs are primarily expressed in phloem, suggesting their involvement in the uptake of cytokinins from xylem sap during long-distance transport. AtPUP1 and AtPUP2 have demonstrated the ability to transport adenine and nucleobase cytokinin derivatives in yeast and Arabidopsis cells. However, with more than 20 PUP genes in the

Arabidopsis genome, further investigations are needed to elucidate the specific functions of each.

In higher plants, the translocation of cytokinin ribosides appears to be mediated by ENTs. Arabidopsis AtENT8 (also known as SOI33) and AtENT3 have been identified as mediators of iP uptake in yeast. In rice, OsENT2 is implicated in the selective transport of cytokinin nucleosides into vascular tissues. Furthermore, studies have identified the involvement of the Arabidopsis ABC transporter subfamily G14 (AtABCG14) in loading tZ-type cytokinins into the xylem for transport from roots to shoots. The expression of AtABCG14 is observed in the pericycle and stellar cells of the root, and loss of its function severely impairs the translocation of tZ-type cytokinins, impacting overall plant growth and development (Nedvěd 2021).

5. Cytokinin signal transduction

The discovery of cytokinins in the 1950s marked the beginning of extensive research on their physiological roles and metabolism. However, understanding cytokinin signal transduction lagged behind, and until the late 1990s, efforts to unravel the signaling mechanisms faced challenges. Initial hypotheses proposed the involvement of calmodulin and a G-protein-linked receptor in cytokinin signaling, but direct evidence was lacking (Suzuki et al 2001). Genetic screens for Arabidopsis mutants with altered responses to exogenously applied cytokinins did not yield significant results due to the challenges posed by the strong influence of cytokinins on the ethylene response and the redundancy of cytokinin signaling components (Hwang et al. 2012).

In the mid-1990s, breakthroughs occurred when Tatsuo Kakimoto and colleagues identified key regulators of cytokinin signaling through genetic screens for mutants with constitutive or impaired cytokinin responses. The positive regulators, CYTOKININ INDEPENDENT 1 (CKI1) and CYTOKININ RESPONSE 1 (CRE1), were identified, along with the discovery of cytokinin-inducible genes, type-A Arabidopsis response regulators (ARRs). These findings paved the way for a more comprehensive understanding of the cytokinin signal transduction cascade (Pischke et al. 2002).

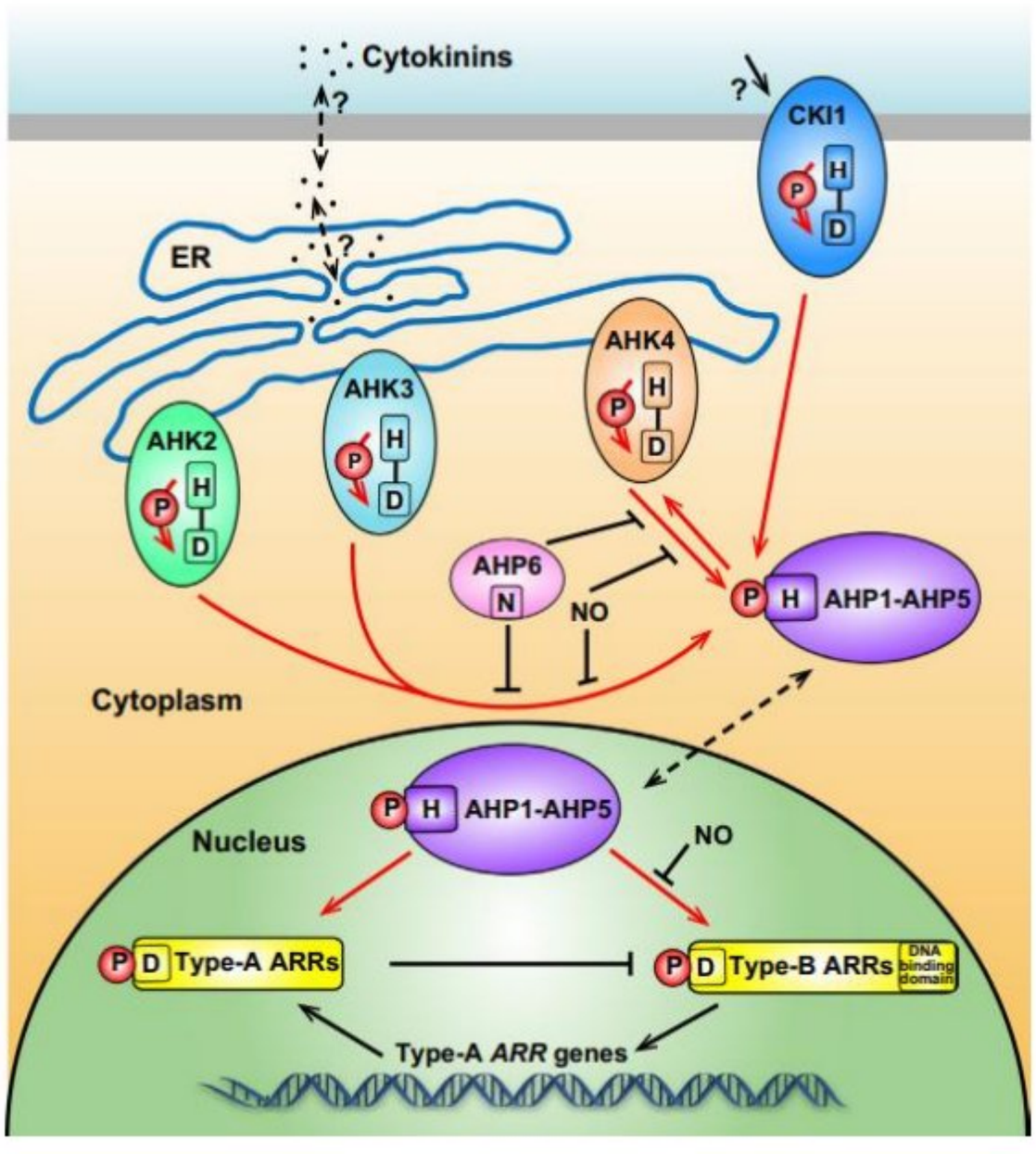

Figure 2. Core steps of the cytokinin signaling pathway. The cytokinin signaling cascade is initiated by cytokinin binding to the cytokinin receptors AHK2, AHK3, and AHK4 within the lumen of the endoplasmic reticulum (ER). After binding to cytokinins, the cytokinin receptors are autophosphorylated at conserved histidine residues in the kinase domain.The

phosphate groups are then transferred to the conserved aspartic acid residues in the receiver domain of the receptors, and then are likely transferred to the histidine residues of AHPI-AHPS in the cytoplasm. The phosphorylated AHPs translocate into the nucleus by an unknown mechanism and transfer the phosphate groups to the conserved aspartic acid residues in the receiver domain of type-A ARRs or type-B ARRs. In the absence of cytokinins, AHK4/CRE1/WOL removes phosphate groups from AHPs. CK/1 also mediates phosphorylation of AHPs in a cytokinin-independent manner. The stability of type-A ARR proteins may be regulated by phosphorylation. The phosphorylated type-B ARRs activate the expression of downstream genes, regulating plant growth and development. Type-B ARRs activate the expression of downstream genes, regulating plant growth and development. Type-B ARRs activate the expression of type-A ARRs, and type-A ARRs, in turn, act to repress the activity of type-B ARRs by a negative feedback mechanism. P denotes the phosphate group: H and D indicate histidine an aspartic acid; black solid circles indicate cytokinins (Feng et al. 2017).

Cytokinin signaling involves a two-component system with a phosphorelay mechanism, mediated by three histidine kinases (AHK2, AHK3, and AHK4) as cytokinin receptors. Upon cytokinin binding, receptor activation involves autophosphorylation and subsequent phosphorylation of downstream components, including Arabidopsis type-B and type-A response regulators (ARRs). The phosphorylated type-B ARRs, which are MYB-class transcription factors, activate the expression of type-A ARR genes and other downstream targets, leading to cytokinin responses. Type-A ARR proteins, in turn, negatively regulate phosphorelay activity, forming a feedback loop. The bidirectional phosphorelay is regulated by the cytokinin receptors and various negative regulators (Tran et al. 2010). This signaling pathway,

composed of AHK receptors, AHPs, and ARRs, has been identified not only in Arabidopsis but also in other plant species like rice, Marchantia polymorpha, and *Physcomitrella patens*, indicating the evolutionary conservation of cytokinin signaling components. The elucidation of the cytokinin signal transduction pathway has provided valuable insights into the molecular mechanisms governing plant growth and development (Fig. 2) (Feng et al. 2017).

6. Functions

Over the last decade, significant strides have been made in unraveling the intricacies of cytokinin, a plant hormone, encompassing its biosynthesis, perception, and signaling pathways. The realization that any disruption in these processes profoundly affects various stages of growth and development has sparked a renewed surge of interest in comprehending the impact of cytokinin signaling on developmental mechanisms. Consequently, recent efforts have yielded fresh insights into the role of cytokinin signaling and its downstream targets in pivotal developmental processes such as shoot apical meristem, flower formation, female gametophyte development, stomatal regulation, and vascular growth. This review aims to provide a comprehensive overview of the latest discoveries regarding how cytokinin influences plant growth and development, shedding light on emerging areas for future research.

6.1 Shoot development

Over the past decade, the exploration of cytokinins in plant biology has revealed their pivotal role in shaping plant growth, particularly when in conjunction with auxin. The collaboration of these hormones, specifically

auxin and cytokinin, has been a central focus of research, unveiling their profound influence on plant development. Experimental observations have demonstrated that plants exposed to heightened levels of auxin and cytokinin undergo extensive proliferation and dedifferentiation, ultimately leading to the formation of callus—a mass of undifferentiated cells. Notably, the cultivation of callus under conditions of elevated cytokinin levels has been linked to the induction of shoot regeneration, firmly establishing cytokinins as key players in the intricate orchestration of shoot development (Su et al. 2011).

The significance of cytokinin signaling extends into the realm of shoot apical meristem (SAM) development, where it has been demonstrated to possess the capability to induce shoot formation in callus. Remarkably, under specific conditions, exogenous cytokinins have been shown to trans-differentiate lateral root primordia into a SAM. This intriguing capability suggests that cytokinins can exercise their influence to specify shoot cell fate across diverse cell types. In the natural context, studies have emphasized the crucial role of cytokinins in SAM formation, as reduced cytokinin levels or compromised signaling pathways result in a discernibly smaller SAM (Kean-Galeno et al. 2024). Transport mechanisms further contribute to the nuanced regulation of cytokinins. ARABIDOPSIS ATP-BINDING CASSETTE G14 (ABCG14) has emerged as a key player in modulating root-derived cytokinin transport. Additionally, the involvement of PURINE PERMEASE 14 (PUP14) transporters has been suggested in mediating the confinement of cytokinins within the SAM. These transport processes underscore the intricacies involved in the spatial regulation of cytokinins, emphasizing their importance in finely tuning developmental processes.

Delving into the molecular intricacies within the SAM, cytokinins act as positive regulators of WUSCHEL (WUS), a gene crucial for stem cell niche maintenance. Recent observations have shed light on the direct binding of B-type ARRs to the promoter region of WUS, thereby promoting its expression. The interplay between WUS and cytokinin signaling emerges as indispensable during shoot specification and regeneration. Mutant explants lacking WUS or specific ARR genes exhibit compromised shoot regeneration capabilities, highlighting the synergistic nature of these pathways (Jha et al. 2020).

The dynamic relationship between cytokinins and WUS unfolds as a finely tuned interdependence, where the expression of WUS precedes morphological changes into a SAM. This suggests that WUS may serve as a direct mediator of cytokinin-induced shoot specification downstream of the cytokinin response pathway. The necessity of normal cytokinin response for proper WUS expression is evident, as disruptions in this pathway result in deficient shoot regenerative capacities (Argueso et al. 2010).

6.2 Flower development

Cytokinins play a crucial role in various stages of flower development, exerting their influence on processes such as carpel regeneration and determinacy, as well as gynoecium development. In loss-of-function lines of B-type ARRs, specifically ARR1 and ARR10, carpel regeneration from callus was impaired. These ARRs were found to bind the AGAMOUS (AG) promoter region, inducing the expression of the carpel identity-defining gene AG. Carpel regeneration was also hindered in AG amiRNA lines, reinforcing the essential role of AG in carpel formation. These findings, although conducted in a carpel-inducing system, suggest that

cytokinin-dependent control of AG expression through ARR1 and ARR10 may also be functional in normal carpel development (Brecht and Rybel 2019).

Unlike the shoot apical meristem (SAM) that continuously divides to generate new tissues, flower meristems have a defined number of flowers before terminating growth. The determinacy of flower meristems is compromised in ag-10 mutants when treated with exogenous cytokinins, resulting in additional tissues within the carpels. ETTIN/AUXIN RESPONSE FACTOR 3 (ARF3) normally restricts cytokinin signaling by repressing IPT, LOG, and AHK genes. Prolonged WUS expression in the arf3-29 mutant suggests that ETTIN/ARF3 repression of cytokinin signaling is crucial for flower determinacy, potentially by limiting WUS expression (Chang et al. 2020).

In gynoecium development, the maximum expression of TCS indicates the involvement of cytokinin signaling. The arr1/10/12 mutant displays fewer ovules, defects in septum fusion, and reduced transmitting tract tissues. Conversely, elevated cytokinin levels lead to over-proliferation of medial tissues. The bHLH transcription factor SPATULA (SPT) influences cytokinin signaling, directly binding to the ARR1 promoter and inducing cytokinin signaling in medial tissues. Cytokinin signaling is confined within medial tissues by inducing the cytokinin inhibitor AHP6, while AG represses cytokinin signaling by inducing A-type ARRs (Hwang et al. 2012).

This intricate interplay of cytokinins, ARRs, and other regulatory factors underscores their multifaceted roles in flower development, not only in the model plant Arabidopsis but also in other species such as Actinidia,

This intricate interplay of cytokinins, ARRs, and other regulatory factors underscores their multifaceted roles in flower development, not only in the model plant Arabidopsis but also in other species such as Actinidia, where a male sex-determining gene, SHY GIRL, encodes a C-type ARR that negatively regulates cytokinin signaling, leading to dioecious flowers. The broad implications of cytokinins in gynoecium development highlight their significance across the plant kingdom (Hong and Fletcher 2023).

6.3 Female gametophyte development

Cytokinins play a pivotal role in the development of ovules within the carpels of the gynoecium, particularly during female gametophyte development. Several cytokinin-associated genes, including cytokinin-insensitive (cki) single mutants, arr7/arr15 double mutants, and ahp2-2/ahp3/ahp5-2 triple mutants, exhibit lethality in female gametophytes. CKI, encoding a histidine kinase activating cytokinin response in the absence of cytokinins, is essential for proper cell fate specification (Brecht and Rybel 2019). In CKI/cki loss-of-function mutants, antipodal and central cells adopt egg cell fate, emphasizing the role of cytokinin signaling. TCS expression is reduced or absent in these cells, further linking cytokinin signaling to cell fate specification. Downstream components AHP1, AHP2, and AHP5 are implicated in the standard cytokinin signaling pathway controlling cell fate.

Contrastingly, CKI overexpression leads to ectopic TCS expression and the specification of the egg cell into a central cell. This misspecification results in the development of a diploid endosperm instead of an embryo upon fertilization. CKI is crucial for providing antipodal and central cell fate, while its repression is necessary for synergid and egg cell specification.

However, the specific mechanisms through which CKI determines these cell fates remain unknown (Yuan et al. 2016).

While this female gametophyte development is not conserved across all plants, as evidenced by gymnosperms lacking central cells and endosperm, the presence of a CKI ortholog suggests its importance in different species. In Ginkgo biloba, the CKI ortholog partially rescues the Arabidopsis cki mutant but fails to confer central cell specification, indicating potential neofunctionalization of CKI during angiosperm evolution, facilitating the formation of central cells and the establishment of endosperm (Brecht and Rybel 2019).

6.4 Root development

Cytokinins, known primarily for their role in shoot development, also play a significant role in root development, as evident from a range of root-related phenotypes observed in biosynthesis, perception, and signaling mutants. The *Arabidopsis* root exhibits clear bilateral symmetry within its vascular tissues, with a central xylem axis flanked by two phloem poles and intervening procambium cells. This bilateral character arises from the tight interplay between auxins and cytokinins. High auxin signaling in xylem cells induces AHP6, repressing cytokinin signaling, while cytokinin signaling in procambial cells affects auxin efflux through PIN-FORMED (PIN) protein expression and localization. Mathematical modeling supports the idea that this interplay is sufficient for achieving bilateral symmetry within the vasculature.

Bilateral symmetry is particularly evident in the pericycle, where lateral roots develop from sets of xylem-pole pericycle cells with high auxin

signaling. AHP6 is implicated in repressing cytokinin signaling in this context. Cytokinins also negatively affect lateral root initiation and organization by disturbing PIN protein localization, thus perturbing local auxin accumulation. This repression of lateral root initiation is crucial for maintaining regular spacing between lateral root primordia (Jing and Strader 2019).

Surprisingly, evidence suggests that bilateral symmetry extends beyond the pericycle into the ground tissue. Endodermal cells at the xylem pole, characterized by higher cytokinin levels, exhibit increased division rates and shorter lengths compared to those neighboring the phloem pole. AHP6 repression of cytokinin signaling in the protoxylem pole endodermis contributes to this bilateral symmetry, guiding correct tissue patterning in collaboration with auxins. In summary, cytokinins play a multifaceted role in root development by influencing vascular tissue symmetry, lateral root initiation, and overall tissue patterning in the root system (Wybouw and De Rybel 2019).

6.5 Cytokinin controls cell divisions in leaf epidermis

Cytokinins play a crucial role in vascular development, as evidenced by classical mutants in the signaling pathway like wooden leg (ahk4/cre1/wol) and ahp6, known for their vascular defects. Recent years have highlighted the significance of the bHLH transcription factor complex, TMO5/LHW, in regulating vascular proliferation, with loss-of-function resulting in diminished vascular cell files and ectopic expression increasing their numbers. This complex binds to the promoter regions of cytokinin biosynthesis genes LOG3 and LOG4 in the xylem axis, suggesting a direct role in radial expansion. Exogenous cytokinin application rescues vascular

bundle size, indicating the essentiality of cytokinins in radial expansion. Perturbations in cytokinin biosynthesis, transport, or signaling lead to reduced vascular cell file numbers, and the induction of CYCLIND3;1 and AINTEGUMENTA is identified as a direct link between cytokinins and cell division. Moreover, cytokinins influence patterning, as mutants exhibit protoxylem identity in all vascular cells, contrasting with TMO5/LHW mutants. The intricate involvement of cytokinins in radial proliferation, patterning, and xylem differentiation in vascular tissues underscores their significance, although the molecular mechanisms underlying these processes remain to be fully elucidated (Wybouw and De Rybel 2019).

6.6 Root nodule formation

The intricate relationship between plants of the Fabaceae family and nitrogen-fixing bacteria is marked by the formation of specialized plant structures known as root nodules, a phenomenon tightly connected to cytokinin signaling. In species like Medicago truncatula and Lotus japonica, the inoculation of nitrogen-fixing bacteria triggers an upregulation in cytokinin biosynthesis and signaling within the affected roots. Notably, in Lotus japonica, triple receptor mutants (lhk1-1 lhk1a-1 lhk3-1) exhibit hyperinfection following Mesorhizobium loti inoculation, yet the infection threads fail to progress into nodule primordia. This observation suggests that cytokinins may not be heavily involved in the initial infection event but rather play crucial roles during the subsequent development of root nodules.

Evidence supporting the importance of cytokinin signaling in nodulation is further underscored by the induction of nodulation factors like MtNSP downstream of cytokinins. This implies that cytokinin signaling is a requisite factor for the nodulation process. Additionally, experiments

involving the overexpression of cytokinin biosynthesis genes demonstrate that root nodule formation can occur even in the absence of nitrogen-fixing bacteria. This finding strongly suggests that cytokinin signaling is not only necessary but also sufficient for the normal development of root nodules, shedding light on the pivotal role played by cytokinins in orchestrating the intricate dance between plants and nitrogen-fixing bacteria in the Fabaceae family (Gamas et al. 2017).

6.7 Vascular development

Cytokinins play a crucial role in vascular development, as evidenced by classical mutants in the signaling pathway like wooden leg (ahk4/cre1/wol) and ahp6, known for their vascular defects (Wybouw and De Rybel 2019). Recent years have highlighted the significance of the bHLH transcription factor complex, TMO5/LHW, in regulating vascular proliferation, with loss-of-function resulting in diminished vascular cell files and ectopic expression increasing their numbers. This complex binds to the promoter regions of cytokinin biosynthesis genes LOG3 and LOG4 in the xylem axis, suggesting a direct role in radial expansion. Exogenous cytokinin application rescues vascular bundle size, indicating the essentiality of cytokinins in radial expansion (Smet et al. 2019). Perturbations in cytokinin biosynthesis, transport, or signaling lead to reduced vascular cell file numbers, and the induction of CYCLIND3;1 and AINTEGUMENTA is identified as a direct link between cytokinins and cell division. Moreover, cytokinins influence patterning, as mutants exhibit protoxylem identity in all vascular cells, contrasting with TMO5/LHW mutants. The intricate involvement of cytokinins in radial proliferation, patterning, and xylem differentiation in vascular tissues underscores their significance, although the

molecular mechanisms underlying these processes remain to be fully elucidated (Campbell and Turner 2017).

7. Conclusion

In conclusion, cytokinins play a pivotal role as plant hormones, exerting significant influence on various physiological processes crucial for plant growth and development. The journey of cytokinins encompasses their synthesis, signal transduction, and diverse functions within the plant system.Cytokinins are primarily synthesized in the roots and transported upwards to different plant organs. The biosynthesis involves isoprenoid or adenylate-type pathways, resulting in the production of various cytokinin forms, including zeatin, isopentenyladenine, and others. Upon synthesis, cytokinins initiate a complex signal transduction pathway, involving receptors, histidine kinases, and phosphorelays. This process triggers downstream responses, influencing gene expression and cellular activities. The functions of cytokinins extend beyond cell division; they participate in leaf senescence, nutrient mobilization, and stress responses. Cytokinins act as key regulators in maintaining the balance between shoot and root growth, ensuring optimal plant architecture. Moreover, they contribute to the coordination of various developmental stages, from seed germination to flower and fruit development. In summary, the multifaceted roles of cytokinins make them indispensable players in the intricate network of plant growth regulation. Their involvement in diverse physiological processes underscores their importance in shaping the overall growth, development, and adaptive responses of plants in their dynamic environment. As our understanding of cytokinins advances, so does the potential for harnessing their regulatory properties to enhance crop yield, stress tolerance, and overall plant productivity.

References

Ahmad N, Jiang Z, Zhang, L, Hussain I, Yang X (2023) Insights on phytohormonal crosstalk in plant response to nitrogen stress: A focus on plant root growth and development. Int J Mol Sci 24(4): 3631.

Argueso CT, Raines T, Kieber JJ (2010) Cytokinin signaling and transcriptional networks. Curr Opin Plant Biol 13(5): 533–539.

Bielach A, Hrtyan M, Tognetti VB (2017) Plants under stress: involvement of auxin and cytokinin. Int J Mol Sci 18(7): 1427.

Brecht W, Rybel BD (2019) Cytokinin–a developing story. Trends Plant Sci 24(2): 177–185.

Bürkle L, Cedzich A, Döpke C, Stransky H, Okumoto S, Gillissen B, Frommer WB (2003) Transport of cytokinins mediated by purine transporters of the PUP family expressed in phloem, hydathodes, and pollen of *Arabidopsis*. Plant J 34(1): 13–26.

Campbell L, Turner S (2017) Regulation of vascular cell division. J Exp Bot 68(1): 27–43.

Chang W, Guo Y, Zhang H, Liu X, Guo L (2020) Same actor in different stages: genes in shoot apical meristem maintenance and floral meristem determinacy in *Arabidopsis*. Front Ecol Evol 8: 89.

Chen CM, Leisner SM (1984) Modification of cytokinins by cauliflower microsomal enzymes. Plant Physiol 75: 442–446.

Chen KL, Xu MX, Li GY (2006) Identification of AtENT3 as the main transporter for uridine uptake in *Arabidopsis* roots. Cell Res 16: 377–388.

Chen S, Lou S, Zhao X, Zhang S, Chen L, Huang P, Li G, Li Y, Liu Y, Chen Y (2022) Ectopic expression of a male fertility gene, LOGL8,

represses LOG and hinders panicle and ovule development. J Crop Sci 10(6): 1665–1673.

Chaudhary, N. (2013). The interaction of cytokinin and strigolactone in controlling shoot branching in *Arabidopsis thaliana* (Doctoral dissertation, Imperial College London).

Fahad S, Hussain S, Bano A, Saud S, Hassan S, Shan D, Ahmed F, Khan F, Chen Y, Wu C, Tabassum M, Chun M, Afsal M, Jan A, Tariq M, Huang J (2015) Potential role of phytohormones and plant growth-promoting rhizobacteria in abiotic stresses: consequences for changing environment. Environ Sci Pollut Res Int 22: 4907–4921.

Feng J, Shi Y, Yang S, Zuo J (2017) Cytokinins. In: Li J, Li C, Smith SM (eds.) Hormone metabolism and signaling in plants. Academic Press, pp 77–106.

Frebort, I, Kowalska, M, Hluska, T, Frébortová, J, Galuszka, P (2011) Evolution of cytokinin biosynthesis and degradation. J Exp Bot 62(8): 2431–2452.

Gamas P, Brault M, Jardinaud M. F, Frugier F (2017) Cytokinins in symbiotic nodulation: when, where, what for? Trends Plant Sci 22(9): 792–802.

Haberer G, Kieber JJ, (2002) Cytokinins. New insights into a classic phytohormone. Plant Physiol 128: 354–362.

Hong L, Fletcher JC (2023) Stem cells: Engines of plant growth and development. Int J Mol Sci, 24(19): 14889.

Hwang I, Sheen J, Müller B (2012) Cytokinin signaling networks. Annu Rev Plant Biol 63: 353–380.

Jha P, Ochatt SJ, Kumar V (2020) WUSCHEL: a master regulator in plant growth signaling. Plant Cell Rep 39: 431–444.

Jing H, Strader LC (2019) Interplay of auxin and cytokinin in lateral root development. Int J Mol Sci 20(3): 486.

Kakimoto T (2001) Identification of plant cytokinin biosynthetic enzymes as dimethylallyl diphosphate: ATP/ADP isopentenyl transferases. Plant Cell Physiol 42: 677–685.

Kean-Galeno T, Lopez-Arredondo D, Herrera-Estrella L (2024) The shoot apical meristem: an evolutionary molding of higher plants. Int J Mol Sci 25(3): 1519

Kieber JJ, Schaller GE (2018) Cytokinin signaling in plant development. Development 145(4): 149344.

Kudo T, Kiba T, Sakakibara H (2010) Metabolism and long-distance translocation of cytokinins. J Integr Plant Biol 52(1): 53–60.

Müller B (2011) Generic signal-specific responses: cytokinin and context-dependent cellular responses. J Exp Bot 62(10): 3273–3288.

Müller D, Leyser O. (2011). Auxin, cytokinin and the control of shoot branching. Ann Bot 107(7): 1203–1212.

Nedvěd D (2021) Transport and metabolism of radio-labelled cytokinins in plant cells and tissues. Int J Mol Sci. 22(7): 3428.

Pischke MS, Jones LG, Otsuga D (2002) An *Arabidopsis* histidine kinase is essential for megagametogenesis. Proc Natl Acad Sci USA 99: 15800–15805.

Sakakibara H (2006) Cytokinins: activity, biosynthesis, and translocation. Annu Rev Plant Physiol 57: 431–449.

Su YH, Liu YB, Zhang XS (2011) Auxin–cytokinin interaction regulates meristem development. Mol Plant 4(4): 616–625.

Suzuki T, Miwa K, Ishikawa K (2001) The *Arabidopsis* sensor His-kinase, AHK4, can respond to cytokinins. Plant Cell Physiol 42: 107–113.

Takei K, Sakakibara H, Sugiyama T (2001) Identification of genes encoding adenylate iso pentenyltransferase, a cytokinin biosynthesis enzyme, in *Arabidopsis thaliana*. J Biol Chem 276: 26405–26410.

Tran LSP, Shinozaki K, Yamaguchi-Shinozaki K (2010) Role of cytokinin responsive two-component system in ABA and osmotic stress signalings. Plant Signal Behav 5(2): 148–150.

Wang X, Ding J, Lin S, Liu D, Gu T, Wu, H, Li Y (2020) Evolution and roles of cytokinin genes in angiosperms 2: Do ancient CKXs play housekeeping roles while non-ancient CKXs play regulatory roles? J Hortic Res 7: 29.

Wybouw B, De Rybel B (2019) Cytokinin–a developing story. Trends Plant Sci 24(2): 177–185.

Yuan L, Liu Z, Song X, Johnson C, Yu X, Sundaresan V (2016) The CKI1 histidine kinase specifies the female gametic precursor of the endosperm. Dev Cell 37(1): 34–46.

Gibberellic Acid: Role in Plant Growth and Development

Deepa P.

PG Department of Botany, Korambayil Ahamed Haji Memorial Unity Women's College, Narukara, Manjeri, Malappuram, 676122, Kerala, India.
Email: deepapsaj@gmail.com

Abstract

Gibberellic acid is a powerhouse plant hormone that plays a starring role in many aspects of plant life from seed germination to towering stalks and juicy fruits. It belongs to the tetracyclic diterpenoid phytohormone family which can combat abiotic and other physiological stresses; and synthesizes in plants from acetyl-CoA by a series of enzymatic actions. Due to the multiple roles in plant growth and development including seed germination, stem elongation, flowering and fruit development, the hormone plays inevitable role in advanced achievements of agriculture, horticulture and tissue culture technology.

Keywords: Gibberellic acid, Phytohormones, Plant development, Plant growth regulators, Seed dormancy, Stress tolerance.

1. Introduction

Gibberellic acid (GA) is a plant hormone that belongs to the gibberellin family and plays significant role in many growth and development processes including stem elongation, cell division and flowering (Fig. 1). It is produced naturally by plants, but it can also be manufactured synthetically and used as a plant growth regulator. Some of the most common natural sources of GA include fungi, bacteria and plants;

while synthetic GA is produced using a fermentation process with the fungus *Gibberella fujikuroi* and then purified and concentrated into a liquid or powder form (Gupta and Chakrabarty 2013). The hormone is used in agriculture to improve crop yield and quality, and also play role in horticulture to produce larger and more attractive flowers and fruits; moreover, the hormone is applicable in brewing to improve the flavour and aroma of beer. There are more than 70 gibberellins isolated and named as GA1, GA2, GA3 and so on in which GA3 is the most widely studied plant growth regulator (Nickerson 1959).

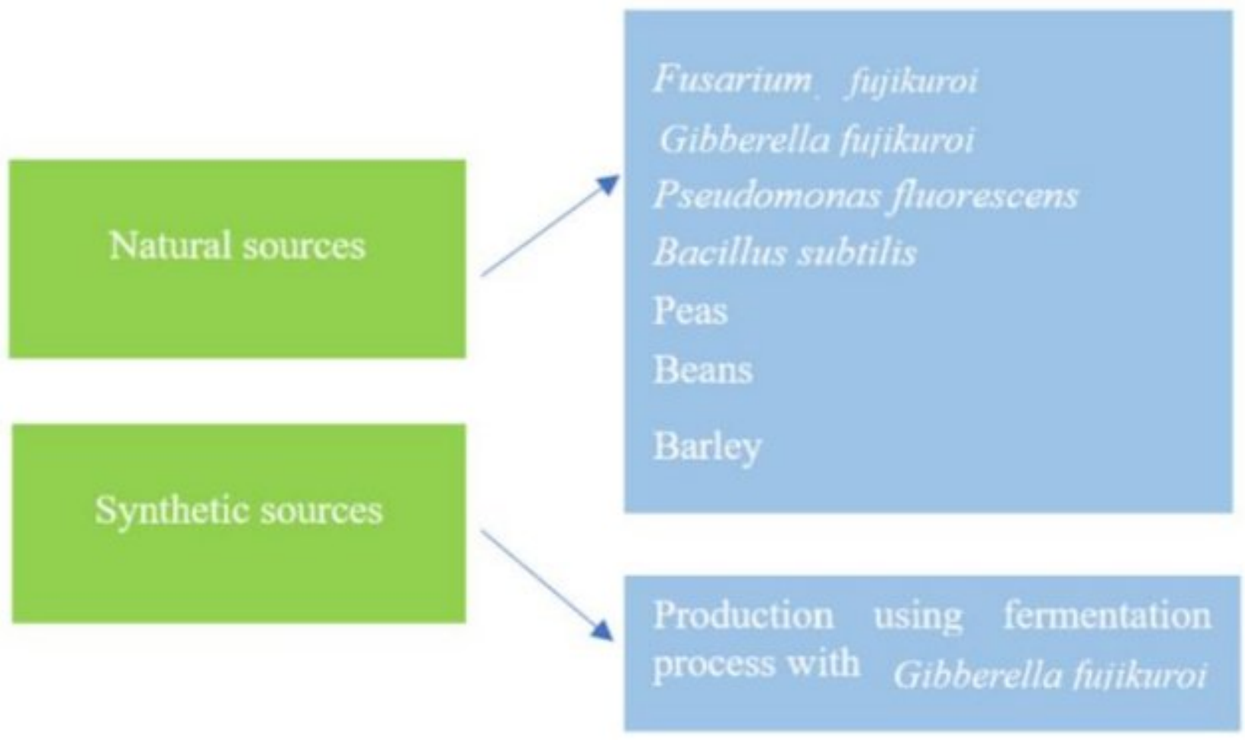

Figure 1. Different sources of GA including plants and microbial organisms

2. Discovery and occurrence

The story of gibberellins wouldn't be complete without delving into the fascinating history of their discovery. The saga begins in the early 1900s, when Japanese rice farmers faced a mysterious disease known as "bakanae," characterized by abnormally tall, slender rice plants with pale leaves and weak stalks. This disease significantly reduced rice yields, posing a major challenge to farmers. To combat this problem, a Japanese scientist named Eiichi Kurosawa embarked on a quest to understand the cause of bakanae disease. He suspected a fungus growing on the rice plants and isolated the

culprit, later identified as *Gibberella fujikuroi*. Kurosawa and his colleagues prepared extracts from the fungus and observed that when applied to healthy rice plants, they exhibited the same tall, spindly characteristics as those infected with the disease. This confirmed that the fungus produced a substance responsible for the abnormal growth. Over the next decade, other Japanese scientists, like Teijiro Yabuta, Bunsuke Sumiki, and Tatsuwo Hayashi, continued the research. They were able to isolate the active substance from the fungal extract and crystallize it in 1935. This newly discovered plant growth regulator was named "gibberellin" after the fungus responsible, *Gibberella fujikuroi*. However, the composition of another compound isolated alongside it, named "gibberellin B," remained unclear (Hedden 2017).

GAs are not just present in a single location within a plant, but rather perform their diverse functions in a widespread and dynamic manner throughout various tissues and organs. GAs are present in meristems of roots, shoots and fruits, where they play a key role in stimulating cell division and elongation, contributing to overall plant growth. Leaves, the photosynthetic engines of plants capture sunlight and converting it into energy. In the organ, GAs are present and influence leaf development including blade expansion, petiole elongation and chloroplast development. GAs can be present in buds, where they play a role in bud break (the transition from dormancy to active growth) and flower development. Fruits, the ripened ovaries of flowering plants, contain seeds and serve as a means of dispersal, in which GAs can be present and influencing fruit cell division and expansion in specific cases.

3. Chemical structure of gibberellic acid

GA is a tetracyclic diterpene acid with a molecular formula of $C_{19}H_{22}O_6$ and having four rings labelled as A, B, C and D, ent-gibberellane ring structure. The A and B rings are fused together; similar to this, the C and D rings are retaining the fusion. The D ring has a carboxylic acid group at position 17, hydroxyl group at position 3 and a methyl group at position 7. Structurally, GA can be categorized based on the number of carbon atoms they contain: either 20-carbon (C20) or 19-carbon (C19) gibberellins. The C20 gibberellins serve as precursors to the C19 gibberellins and inherently lack bioactivity (Fig. 2). For a C19 gibberellin to be bioactive, it must possess a 3β-hydroxyl group and lacks a 2β-substituent (Mulholland and Ward 1954).

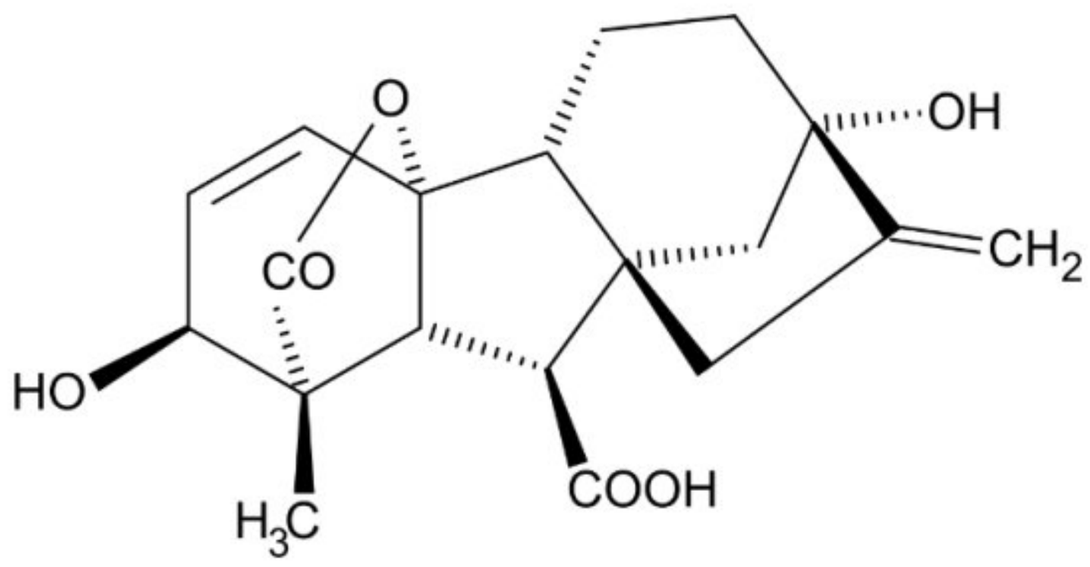

Figure 2. Structure of GA3 with four different rings A, B, C and D

4. Types of plant originated gibberellic acid

More than hundred gibberellic acids are reported till date; among them, the major bioactive growth regulators including GA1, GA3, GA4 and GA7, have a hydroxyl group on C-3β, a carboxyl group on C-6 and a lactone between C-4 and C-10 (Nickerson 1959).

- Gibberellic acid A1 (GA1): The plant hormone plays a crucial role in various aspects of growth and development including seed germination, stem elongation, leaf expansion, flowering and fruit

development. The tetracyclic diterpenoid acid is synthesized in various plant tissues like young leaves, developing seeds and embryos.

- Gibberellic acid A3 (GA3): The most common type of gibberellic acid that used in a variety of agricultural and horticultural applications, stimulates plant growth, increases fruit size and delays senescence.
- Gibberellic acid A4 (GA4): Type of gibberellic acid is less common than GA3, but it is still used in some agricultural applications, mainly to promote flowering and fruit set.
- Gibberellic acid A7 (GA7): It is the least common type GA and not as well-studied as GA3 and GA4 which shows some effects on plant growth, but its specific effects are not fully understood.

5. Gibberellic acid biosynthesis

GA is synthesized in plants through a complex pathway that involves several enzymes. The starting material for GA synthesis is acetyl-CoA in turn converted to geranylgeranyl pyrophosphate (GGPP) by a series of enzymes. GGPP is then cyclized to form ent-kaurene and further oxidized and modified to form GA (Fig. 3). Moreover, GA can be synthesized chemically; but this is a complex and expensive process in which the most common method of chemical GA synthesis involves the oxidation of ent-kaurene with potassium permanganate (Corey and Munroe 1982).

Stage 1. Cyclization of GGPP: The synthesis commences with the cyclization of GGPP (geranylgeranyl diphosphate) to produce the fully cyclized compound, ent-kaurene. This process involves two sequential enzymes: copalyl diphosphate synthase (CPS) and ent-kaurene synthase (KS) which are localized within plastids (Corey and Munroe 1982). *Stage 2.*

Oxidation processes: Ent-kaurene undergoes a series of oxidations at the C-19 position, culminating in the formation of ent-7a-hydroxykaurenoic acid. The compound is then transformed into GA12-aldehyde through B ring contraction and further oxidation at C-6. The enzymes responsible for these oxidations including ent-kaurene oxidase and ent-kaurenoic acid hydroxylase, are membrane-bound cytochrome P450 monooxygenases that are typically associated with the endoplasmic reticulum (Corey and Munroe 1982). *Stage 3. Diverse pathways*: From GA12-aldehyde, the biosynthesis can diverge into multiple pathways; some of which are species-specific. Common steps include the oxidation of GA12-aldehyde to GA12, subsequent oxidations at C-20 and lactone formation. Depending on the plant species, GA12 can be hydroxylated at C-13 to produce GA53, which is then converted to GA20 and GA1. Alternatively, GA12 can lead to the formation of GA9 and GA4. The specific pathway is likely under developmental control, ensuring the synthesis of this crucial hormone remains flexible and adaptable (Corey and Munroe 1982).

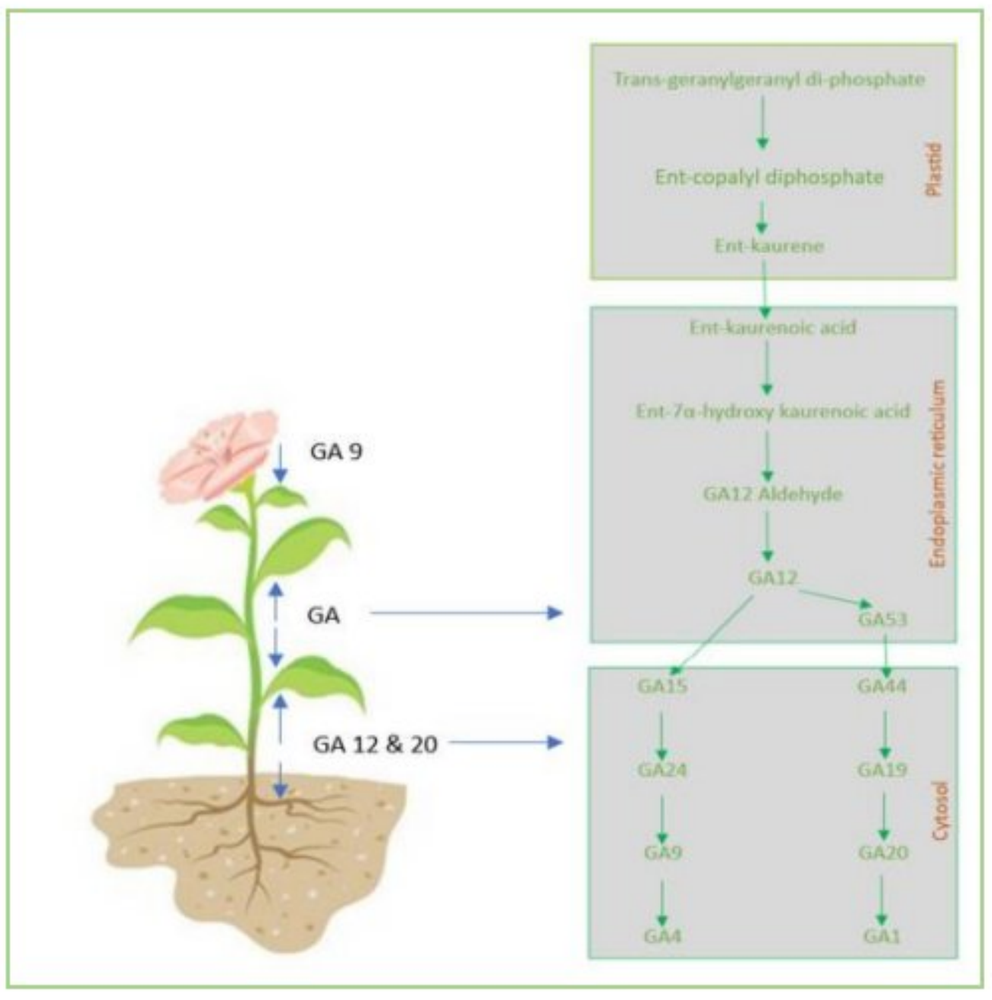

Figure 3. Steps of GA synthesis in developing plants

6. Role of gibberellic acid in plant development

In addition to the key functions, GA plays major role in other aspects of plant growth and development such as leaf expansion, root growth and stress tolerance. Normally, GA is produced in the leaves and young stems of plants and then transported to other parts of the plant where it acts on target cells. GA binds to specific receptors on the cell membrane, in turn activates a signal transduction pathway that leads to changes in gene expression (Tanimoto 2005). GA promotes stem elongation by stimulating cell division; hence, the plants treated with GA often grow taller than untreated plants. In many plants such as dwarf pea and maize, the genetic dwarfism can be overcome by internode elongation which is the most pronounced effects of gibberellins on plant growth (Vince 1967). GA promotes fruit development by stimulating cell division in turn cause enlargement of the fruit. However, the fruits treated with GA often grow larger than untreated fruits (Zang et al. 2016). The buds that are formed in autumn stay dormant until next spring. By treating with GA, the dormancy can be overcome and result in better shooting (Zheng et al. 2018).

7. Roles of gibberellic acid in modern technology

7.1 Plant tissue culture technology

GA is probably used in plant tissue culture to promote cell division and growth that resulting the increased regeneration of shoots and roots from explants. It can be also promoted the development of somatic embryos from somatic cells of explant. GA is often used in combination with other plant hormones such as auxin and cytokinin to optimize the growth and development of plant cultures (Fig. 4 and 5).

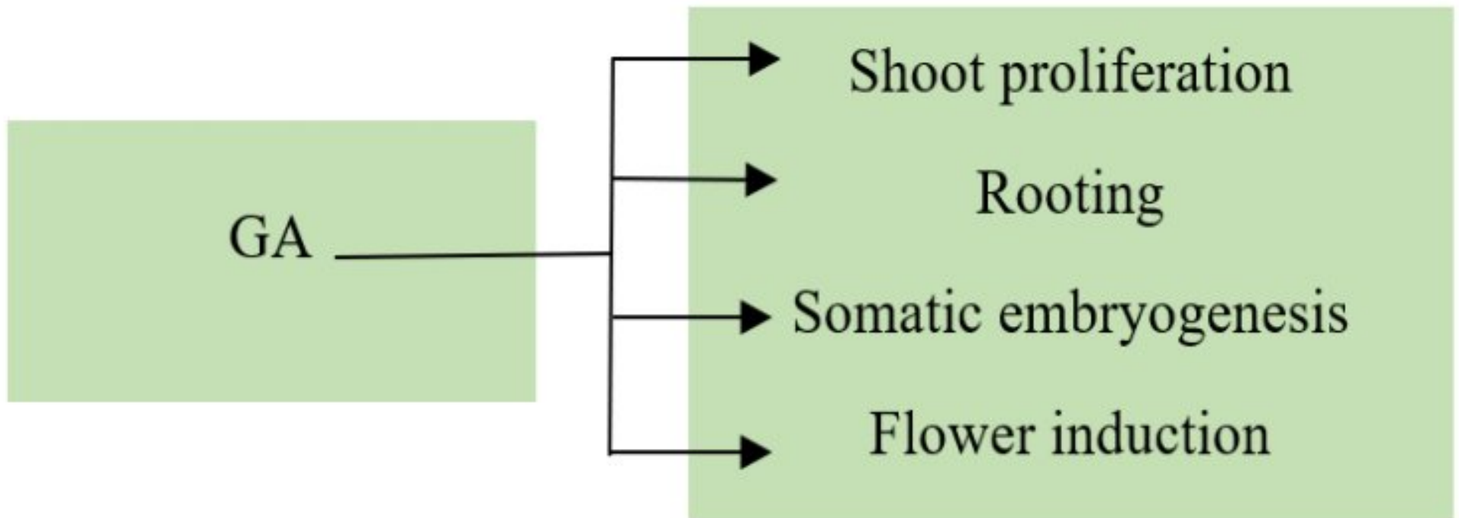

Figure 4. Role of GA in *in vitro* regeneration of plantlets under aseptic conditions.

Following are some specific examples of how GA is used in plant tissue culture (Mahmoody and Noori 2014).

1. Supplementation of GA increases the number of shoots produced by explants in plant tissue culture. This is useful for propagating plants that are difficult to propagate by conventional methods like seeds or cuttings (Gupta and Chakrabarty 2013).

2. GA can be promoted the development of roots on explants that are difficult to root from cuttings such as woody plants (Tanimoto 2005).

3. GA induces the *in vitro* development of somatic embryos which develops from somatic cells rather than from gametes in turn producing large numbers of identical plants which can be used for research or commercial purposes (Li and Qu 2002).

4. In tissue cultured plants, GA induces flowering and increases the number of flowers produced. It promotes flowering by stimulating the production of ethylene, another plant hormone that involved in flower initiation; moreover, GA directly promotes the expression of genes that involved in flowering. In addition to promoting the flowering, GA increases the size and number of flowers by enhancing the cell division and elongation in the flower buds. In tissue culture, GA promotes flowering in plants that are difficult to flower or that have long flowering times. Moreover, the method can also be used to

increase the number of flowers produced for cut flower production. Similar to this, GA promotes flowering in orchids, roses, fruit crops etc. which can be difficult to flower in tissue culture (Brian 1958).

Figure 5. *In vitro* flowering, shoot proliferation and rooting in carnation plant by the supplementation of GA as growth regulator.

7.2 Agriculture

GA plays a vital role in a variety of agricultural processes including seed germination by breaks down the seed coat and allowing the embryo to emerge, stem elongation by promoting the cell growth and division, flowering by stimulating the production of flower buds and fruit development by enhancing the fruit quality (Gupta and Chakrabarty 2013). The hormone application improves the crop yield and quality by increasing the number of tillers in wheat and rice, increasing the size of grapes and berries, reducing pre-harvest fruit drop in citrus and apples and delaying senescence in leafy vegetables. It can be applied to crops in a variety of ways including foliar sprays, seed treatments and soil applications. The optimal

application method and rate will vary depending on the crop and the desired effect. In agriculture, GA used for different purposes including 1. *Seed germination*: GA improves the germination rate of seeds that have been stored for a long period of time or that have been damaged, 2. *Stem elongation*: Hormone produces taller plants in crops such as sugarcane and bamboo, 3. *Flowering*: It stimulates flowering in crops such as mangoes and pineapple, and 4. *Fruit development*: GA increases the size of fruits in crops such as grapes and berries. Hence, the plant hormone is powerful that can be used to improve crop yields and quality in a variety of ways. However, it is important to use GA carefully and according to the instructions on the product label (Pereira et al. 2019).

7.3 Floriculture

In floriculture, GA plays a role in many aspects of plant growth and development by promoting stem elongation, flower initiation and flower development in responsible to the application in plants as foliar spray, soil drench or seed treatment. The specific method and the rate of application will vary depending on the desired effect in a variety of floriculture crops including roses, carnations and lilies. GA can also be used to increase the number of flowers produced by some crops such as chrysanthemums, poinsettias, orchids and tulips. Similar to this, the hormone delays flower senescence in some crops like cut flowers. Normally, farmers are used GA to increase the stem length of roses, flower size of carnations, flower initiation in lilies, promote flower development in orchids and delay flower senescence in cut flowers. Hence, the treatment is a useful tool that can be used to improve the quality and quantity of flowers produced in floriculture. However, it is important to use GA carefully, as it can also have negative effects on plants if it is not used correctly (Pradeepkumar et al. 2020).

Table 1. Different roles of GA in plant growth and development.

Role of GA in plant development	
Promoting plant growth	GA can be applied to seeds, seedlings or mature plants to promote growth of potted and budding plants mainly for increasing their size and vigour (Brian et al. 1954).
Increasing yields	The hormone can be used to increase yields of many crops including fruits, vegetables and flowers. Mainly, it is used to increase the size and number of grapes, apples and citrus fruits in addition to increase the number of blooms on roses and other flowering plants (Ramesh et al. 2019).
Improving crop quality	The growth regulator improves the quality of many crops by changing the metabolic activities. It improves the colour and flavour of tomatoes and strawberries, and also increases the shelf life of fruits and vegetables (Miceli et al. 2019).
Breaking seed dormancy	Phytohormone breaks seed dormancy in some crops, such as lettuce and beets. This allows growers to plant seeds earlier in the season and extend the harvest season (Lee et al. 2016).
Delaying flowering	GA delays flowering in some crops like *Chrysanthemum* sp. and *Poinsettia* sp. which allows growers to produce flowers for specific holidays or markets (Saks et al. 1992).

7.4 Horticulture

In horticulture, GA is used to promote plant growth, increase yields and improve crop quality by breaking seed dormancy, delaying flowering and extending the shelf life of fruits and vegetables. However, it is important to use GA according to label directions as excessive use can have negative effects on plant growth and development (Bagale et al. 2022).

8. Gibberellic acid production under abiotic stress conditions

GA plays a major role in plant growth and development processes including seed germination, stem elongation, leaf expansion, flower initiation and fruit development (Fig. 6). In most cases, the hormone production in plants is influenced by environmental stresses. Under environmental stress such as drought, salinity or heat, GA production is often reduced by varying the metabolic rates, mainly by inhibiting the activity of the enzymes involved in GA biosynthesis. Additionally, stress conditions can be induced the production of other plant hormone like abscisic acid (ABA) which can antagonize GA signalling. Despite the fact that GA production is often reduced under environmental stress, it can still play an important role in helping plants to cope with stress. Obviously, it can help to maintain cell division and elongation which can promote plant growth even under stress conditions by regulating the expression of genes involved in stress tolerance viz. genes involved in osmo-protectant synthesis and antioxidant defence (Mahmoody and Noori 2014).

Drought stress normally reduces GA production in a number of ways and inhibits the activity of the enzymes involved in GA biosynthesis. At the same time, drought can be induced the production of ABA which can antagonize GA signalling. Sometimes, drought leads to the accumulation of reactive oxygen species (ROS), that damage the enzymes involved in GA biosynthesis (Litvin et al. 2016). Similarly, the salinity stress reduces GA production by inhibiting the activity of the enzymes involved in hormone biosynthesis. Additionally, salinity stress leads to the accumulation of Na^+ ions in the plant tissues in turn inhibits GA biosynthesis. Additionally, the heat stress also induces the production of ABA that antagonizes GA

signalling, in turn reduces GA production by denaturing the enzymes involved in biosynthetic pathway (Guo et al. 2022).

Figure 6. Role of GA in different developmental stages of plants.

Despite the fact that GA production is often reduced under environmental stress, it can still play an important role in helping plants to cope with stress. Therefore, it is important to understand how GA production is affected by environmental stress in order to develop strategies for improving plant stress tolerance. One way to improve plant stress tolerance is to apply GA exogenously. Exogenous hormone application helps to maintain cell division and elongation, and regulates the expression of genes involved in stress tolerance. Together with the fact, some studies have shown that exogenous GA application can help to reduce the accumulation of ROS and Na^+ ions in plant tissues under environmental stress. Another way to improve plant stress tolerance is to identify and overexpress genes involved in biosynthetic pathway. This approach has been shown to be effective in improving plant tolerance to drought, salinity and heat stress. By understanding how GA production is affected by environmental stress and by developing strategies to improve GA production under stress conditions, we

can help plants to better cope with environmental challenges and improve crop yields (Fahad et al. 2015).

9. Technologies for enhanced gibberellic acid production in plants

There are a number of technologies that can be used to enhance the production of GA for improving the cultivation practices. Gene editing technologies such as CRISPR-Cas9 can be used to make precise changes to the GA genome which enhances the yield and quality of GA as well as to make GA more resistant to pests and diseases. *Marker-assisted selection* (MAS) uses DNA markers to identify individuals with desired traits and used to accelerate the breeding process for GA as it allows breeders to select for desired traits without having to grow and phenotype large populations of plants. Proteomics is also used to identify and characterize proteins that are involved in GA production which helps to develop new strategies for GA production enhancement (Cho 2007). Metabolomics focuses the identification and characterization of metabolites that involved in GA production in turn helps to develop new strategies for enhancing GA production. In addition to these technologies, there are a number of other factors that can be used to enhance GA production that including 1. *Improved agricultural practices:* Improved agricultural practices such as crop rotation, fertilization and irrigation can help to improve the yield and quality of GA. 2. *Pest and disease management:* Effective pest and disease management practices protect GA crops from pests and diseases which can improve the yield and quality of GA. *3. Post-harvest handling:* Proper post-harvest handling of GA preserves the quality of GA and extend its shelf life (Liu and Locasale 2017; Camara et al. 2018).

By using a combination of these technologies and factors, it is possible to significantly enhance the production of GA. Some of the specific

examples show how these technologies are being used to enhance GA production. 1. Researchers at the University of California, Davis, are using CRISPR-Cas9 to develop GA strains that are resistant to the fungus *Fusarium oxysporum*, which causes a devastating disease in GA crops (Shi et al. 2019). 2. Scientists at the Chinese Academy of Sciences are using MAS to breed GA strains with higher yields and improved quality (Nimisha et al. 2013). 3. Researchers at the University of Guelph use proteomics to identify and characterize proteins that are involved in GA production. This information is being used to develop new strategies for enhancing GA production (Staszak et al. 2018). 4. Scientists at the University of California, Berkeley, are using metabolomics to identify and characterize metabolites that are involved in GA production, indirectly used to develop new strategies for enhancing GA production (Brisson et al. 2021). As technology continues to develop, we can expect to see even more innovative and effective ways to produce GA (Rodrigues et al. 2012).

10. Signal transduction

GA, though a potent plant hormone, doesn't directly exert its influence within the plant. Instead, it relies on a sophisticated signal transduction pathway to translate its presence into an orchestrated response. This pathway involves a series of intricate steps, akin to a well-rehearsed dance, transforming the GA signal into specific cellular actions that ultimately influence growth and development.

Perception: Specialized plant proteins called GIBBERELLIN INSENSITIVE DWARF 1 (GID1) receptors act as the initial point of contact. When an active GA molecule encounters a GID1 receptor, it binds to it, initiating the signalling cascade. *The GID2 Complex Takes Center Stage*:

Upon binding, the GID1 receptor undergoes a conformational change, and interacts with an additional protein complex called SLEEPY1 (SLY1) - GID2**.** This complex plays a crucial role in relaying the GA signal further downstream. *Degradation of DELLA Proteins*: Interestingly, the GID2 complex targets specific proteins within the cell called DELLA proteins. These proteins act as negative regulators of growth**,** repressing various growth-promoting processes. When the GID2 complex is activated by the GA-GID1 complex, it triggers the ubiquitination of DELLA proteins. Ubiquitination essentially marks them for degradation by the plant's cellular machinery. *Growth Takes Flight:* With the removal of DELLA proteins, the stage is set for growth to occur. Various growth-promoting factors, previously inhibited by DELLA proteins, are now free to exert their influence. This can involve in increased gene expression and activation of enzymes. *A Balancing Act*: GA signalling pathway is not a one-way street in which the plants possess intricate mechanisms to control the duration and intensity of the GA response. This includes regulation of GID1 receptor availability and modification of DELLA proteins (Ashikari et al. 2003).

11. Conclusion

Gibberellic acid (GA) stands tall as a leading player in the world of plant hormones, influencing various aspects of plant life from seed germination to majestic stalks and luscious fruits. It belongs to the tetracyclic diterpenoid phytohormone family**,** renowned for its ability to combat various stresses and its synthesis within plants through a series of enzymatic reactions. While over 70 different gibberellins have been identified and named, with GA3 being the most thoroughly studied plant growth regulator,

each GA plays a specific role in the intricate symphony of plant development.

References

Ashikari M, Hironori I, Miyako UT, Sasaki A, Gomi K, Kitano H, Matsuoka M (2003) Gibberellin signal transduction in rice. J Plant Grow Reg 22: 141–151.

Bagale P, Pandey S, Regmi P, Bhusal S (2022) Role of plant growth regulator Gibberellins in vegetable production: an overview. Int J Hortic Sci Techn 9(3): 291–299.

Brian PW (1958) Gibberellic acid: A new plant hormone controlling growth and flowering. J Roy Soc Art 106(5022): 425–441.

Brian PW, Elson GW, Hemming HG, Radley M (1954) The plant-growth-promoting properties of gibberellic acid, a metabolic product of the fungus *Gibberella fujikuroi*. J Sci Food Agr 5(12): 602–612.

Brisson V, Mayali X, Bowen B, Golini A, Thelen M, Stuart RK, Northen TR (2021) Identification of effector metabolites using exo-metabolite profiling of diverse microalgae. Syst 6(6): e0083521.

Camara MC, Vandenberghe LP, Rodrigues C, de Oliveira J, Faulds C, Bertrand E, Soccol CR (2018) Current advances in gibberellic acid (GA3) production, patented technologies and potential applications. Planta 248: 1049–1062.

Chen SS, Chang JL (1972) Does gibberellic acid stimulate seed germination via amylase synthesis? Plant Physio 49(3): 441.

Cho WC (2007) Proteomics technologies and challenges. Gen Prot Bioinf 5(2): 77–85.

Corey EJ, Munroe JE (1982) Total synthesis of gibberellic acid. A simple synthesis of a key intermediate. J Am Chem Soc 104(22): 6129–6130.

Fahad S, Nie L, Chen Y, Wu C, Xiong D, Saud S, Huang J (2015) Crop plant hormones and environmental stress. Sust Agr Rev 15: 371–400.

Guo T, Gull S, Ali MM, Yousef AF, Ercisli S, Kalaji H, Ghareeb RY (2022) Heat stress mitigation in tomato (*Solanum lycopersicum* L.) through foliar application of gibberellic acid. Sci Rep 12(1): 11324.

Gupta R, Chakrabarty SK (2013) Gibberellic acid in plant: still a mystery unresolved. Plant Sign Behav 8(9): e25504.

Hedden P (2017) Encyclopedia of Applied Plant Sciences. 2: 2017

Lee JW, Kim YC, Kim JU, Jo I, Kim KH, Kim DH (2016) Effects of gibberellic acid and alternating temperature on breaking seed dormancy of Panax ginseng CA Meyer. Korean J Med Crop Sci 24(4): 284–293.

Li L, Qu R (2002) In vitro somatic embryogenesis in turf-type bermudagrass: roles of abscisic acid and gibberellic acid and occurrence of secondary somatic embryogenesis. Plant Breed 121(2): 155–158.

Litvin AG, van Iersel MW, Malladi A (2016) Drought stress reduces stem elongation and alters gibberellin-related gene expression during vegetative growth of tomato. J Am Soc Hortic Sci 141(6): 591–597.

Liu X, Locasale JW (2017) Metabolomics: a primer. Trends Biochem Sci 42(4): 274–284.

Mahmoody M, Noori M (2014) Effect of gibberellic acid on growth and development plants and its relationship with abiotic stress. Int J Farm Sci 3(6): 717–721.

Miceli A, Moncada A, Sabatino L, Vetrano F (2019) Effect of gibberellic acid on growth, yield, and quality of leaf lettuce and rocket grown in a floating system. Agron 9(7): 382.

Mulholland TPC, Ward G (1954) Gibberellic acid. Part II. The structure and synthesis of gibberene. J Chem Soc, London, 4676–4681.

Nickerson NH (1959) Sustained treatment with gibberellic acid of five different kinds of maize. Ann Missouri Bot Gard 46(12): 19–37.

Nimisha S, Kherwar D, Ajay KM, Singh B, Usha K (2013) Molecular breeding to improve guava (*Psidium guajava* L.): current status and future prospective. Sci Hortic 164: 578–588.

Pereira ADES, Oliveira HC, Fraceto LF (2019) Polymeric nanoparticles as an alternative for application of gibberellic acid in sustainable agriculture: a field study. Scient Rep 9(1): 7135.

Pradeepkumar CM, Chandrashekar SY, Kavana GB, Supriya BV (2020) A review on role and use of gibberellic acid (GA3) in flower production. Int J Chem Stud 8(1): 3076–3084.

Ramesh S, Sudhakar P, Elankavi S, Suseendran K, Jawahar S (2019) Effect of gibberellic acid (GA3) on growth and yield of rice (*Oryza sativa* L.). Plant Arch 19(1): 1369–1372.

Rodrigues C, Vandenberghe LPDS, de Oliveira J, Soccol CR (2012) New perspectives of gibberellic acid production: a review. Crit Rev Biotech 32(3): 263–273.

Saks Y, Van Staden J, Smith MT (1992) Effect of gibberellic acid on carnation flower senescence: evidence that the delay of carnation flower senescence by gibberellic acid depends on the stage of flower development. Plant Growth Regul 11: 45–51.

Shi TQ, Gao J, Wang WJ, Wang KF, Xu GQ, Huang H, Ji XJ (2019) CRISPR/Cas9-based genome editing in the filamentous fungus

Fusarium fujikuroi and its application in strain engineering for gibberellic acid production. ACS Synth Biol 8(2): 445–454.

Staszak AM, Rewers M, Sliwinska E, Klupczyńska EA, Pawłowski TA (2018) DNA synthesis pattern, proteome, and ABA and GA signalling in developing seeds of Norway maple (*Acer platanoides*). Funct Plant Biol 46(2): 152–164.

Tanimoto E (2005) Regulation of root growth by plant hormones-roles for auxin and gibberellin. Crit Rev Plant Sci 24(4): 249–265.

Vince D (1967) Gibberellic acid and the light inhibition of stem elongation. Planta 75: 291–308.

Zang YX, Chun IJ, Zhang LL, Hong S B, Zheng WW, Xu K (2016) Effect of gibberellic acid application on plant growth attributes, return bloom, and fruit quality of rabbit-eye blueberry. Sci Hortic 200: 13–18.

Zheng C, Kwame Acheampong A, Shi Z, Halaly T, Kamiya Y, Ophir R, Or E (2018) Distinct gibberellin functions during and after grapevine bud dormancy release. J Exp Bot 69(7): 1635–648.

An Insight into Functions and Signal Transduction of Abscisic Acid

Nahla Zakariya P.

PG Department of Botany, Korambayil Ahamed Haji Memorial Unity Women's College, Manjeri, Malappuram, 676122, Kerala, India.

Email: nahlazakariya8@gmail.com

Abstract

Abscisic acid is one of the most important plant growth inhibitors rather than a stimulatory hormone. ABA synthesis occurs in cells containing chloroplasts or amyloplasts. Despite its name reference to abscission, its role extends far beyond that function. The chemical structure of ABA consists of 15 carbon atoms, and its synthesis occurs through two pathways: direct synthesis from mevalonic acid and indirect synthesis from the oxidation of carotenoids within the chloroplast. ABA functions encompass diverse roles, including stomatal regulation during water stress, influencing root and shoot growth, regulating dormancy and germination in seeds, impacting fruit growth and ripening, and participating in senescence regulation. Understanding ABAs signal transduction involves challenges, with multiple putative receptors identified (ABAP1, CHLH, FCA, GCR2) and its involvement in gene transcription, Ca^{2+} signaling, and protein phosphorylation highlighted. Yet, the precise mechanism and interplay with various stress signals remains a subject of ongoing research.

Key words: Abscisic acid, Dormin, Stomatal closure, Stress hormone.

Chapter 5: An Insight into Functions and Signal Transduction of Abscisic Acid

1. Introduction

Abscisic acid is one of the major plant hormones that regulate many aspects of plant growth and development. ABA is ubiquitous in plants. It is also produced by some Phytopathogenic fungi, bacteria and metazoans (Nambara and Marion-Poll 2005). Despite of the name, ABA does not appear to control abscission directly; the presence of ABA in abscising organs reflects its role in promoting senescence or stress responses. This chapter explains the current understanding of ABA structure, biosynthesis, functions and signal transduction.

2. Discovery and occurrence

In 1940 Torsten Hemberg reported that dormant potato tubers and buds of ash (*Fraxinus excelsior*) contained inhibitor that hindered the affect of IAA (Hemberg 1949a, 1949b). Upon germination of the buds, the concentration of these inhibitors decreased. Eagles and Wareing (1963) isolated an inhibitor from the birch (*Betula pubescens*) leaves held under short day conditions. When this substance was reapplied to the leaves of birch seedlings, apical growth was completely arrested. As this substance induce dormancy, they named its as 'dormin' (Eagles and Wareing 1963).

During the same period, Frederick Addicott discovered a substance that regulated the abscission of cotton fruits, and named abscisin II (Ohkuma et al. 1963). Cornforth and his associates demonstrated that both Dormin and Abscissin II where chemically identical and given a common name Abscisic Acid (Cornforth et al. 1965; Addicott et al. 1968). Abscisic acid is a

misnomer for this compound, because it has little to do with abscission. It is mostly found in monocots, dicots, gymnosperms and some ferns. Various genera of fungi produce ABA as a secondary metabolite. In plants ABA is synthesized in almost all cells containing chloroplast and amyloplast, and its presence has been detected in every living tissue, ranging from root caps to apical buds.

3. Chemical structure of ABA

ABA is a sesquiterpene consisting of 15 carbon atoms (Fig. 1). It is unique among plant hormones in having an asymmetric carbon atom. Its structure comprises of six carbon rings with an attached side chain. Because of the asymmetric carbon atom (carbon- 1), it occurs in two enantiomorphic forms, R-abscisic acid and S-abscisic acid. The naturally occurring form is S-abscisic acid. In contrast to auxin, gibberllins and cytokinins, which are represented by various active derivatives, ABA is a single compound.

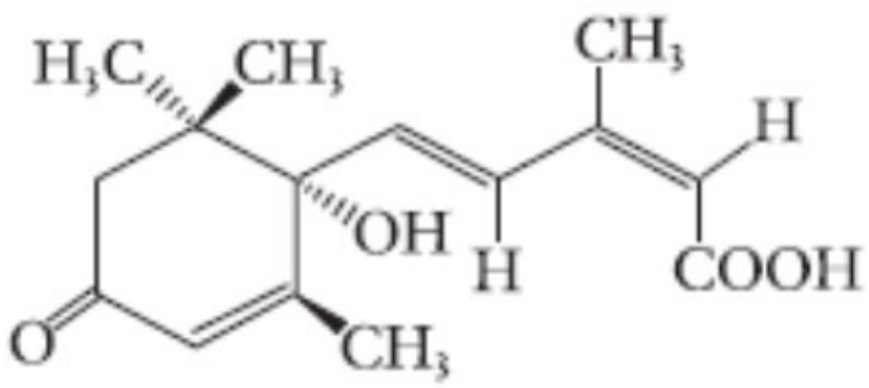

Figure 1. Chemical structure of abscisic acid

4. Biosynthesis of ABA

Two pathways for the biosynthesis of ABA have been identified:

4.1 By direct synthesis from Mevalonic acid

Direct synthesis of ABA from mevalonic acid through farnesyl pyrophosphate has been demonstrated in many cases, especially in water stressed tissues (Hirai et al. 2000; Izquierdo Bueno et al. 2018; Takino et al. 2018). The water stress increases the ABA formation.

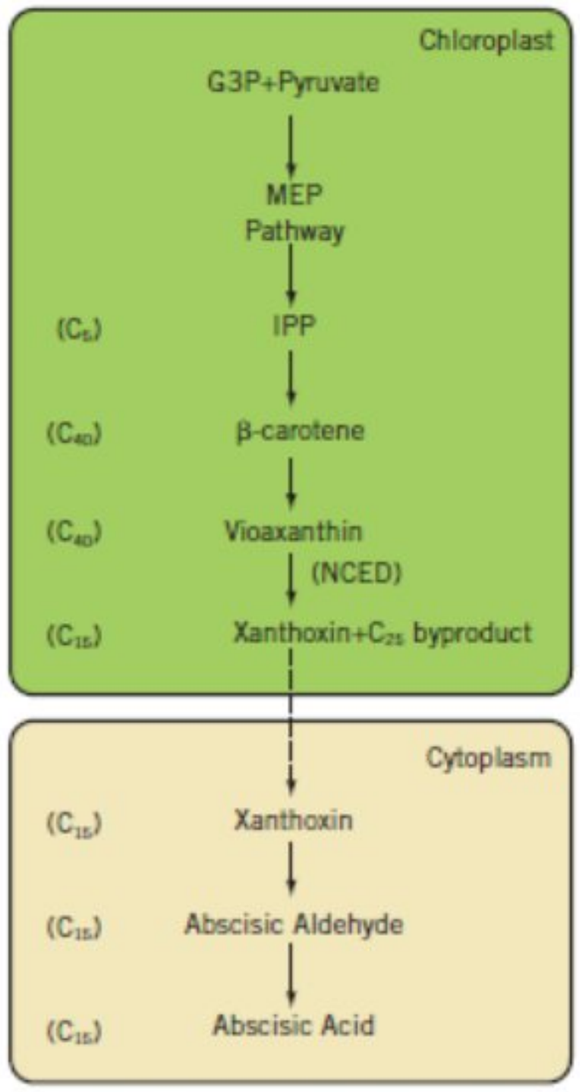

Figure 2. Biosynthesis of ABA from oxidation of carotenoids. G3P- glyceraldehyde 3 phosphate, MEP- methyl erythritol-4-phosphate, IPP- isopentyl diphosphate, NCED- 9'– cis – epoxycarotenoid dioxygenase.

4.2 By indirect synthesis from oxidation of carotenoids

In the chloroplast, isopentyl diphosphate (IPP) is synthesized from glyceraldehyde 3 phosphate and pyruvate via the methyl erythritol-4-phosphate (MEP) pathway. IPP is then converted in to zeaxanthin. Later violaxanthin is synthesized from zeaxanthin in a reaction that is catalysed by the enzyme zeaxanthin epoxidase (ZEP). Violaxanthin is converted in to 9' – cis – neoxanthin which is then converted in to xanthoxal (previously called xanthoxin) by the enzyme 9' – cis – epoxycarotenoid dioxygenase (NCED).

Xanthoxal is then carried to the cytoplasm and converted to ABA aldehyde by the enzyme short chain dehydrogenase (SDR). Finally, ABA aldehyde is converted in to ABA by the enzyme abscisic acid oxidase (AAO) (Nambara and Marion-Poll 2005; Arc et al. 2013) (Fig. 2).

5. Functions

5.1 Stomatal closure

Stomata are pivotal for gas exchange and transpiration of plants, and the closure of stomata can be induced by numerous environmental factors such as drought, pathogen attack, darkness, low humidity, high CO_2 concentrations and so on (Bauer et al. 2013; Assmann and Jegla 2016; Martin et al. 2017; Su et al. 2017). During water stress, ABA concentration increases dramatically in plant leaves, which leads to stomatal closure. Stomatal closure is driven by a reduction in guard cell turgor pressure caused by a large efflux of potassium ion and anions (such as chloride and malate ions) from the cells. Higher ABA concentration in cell increases the cytosolic calcium concentration. ABA stimulates the elevation in the concentration of cytosolic calcium in two ways:

1. By influx of calcium ions through plasma membrane.
2. By release of calcium ions into the cytosol from internal compartments such as Endoplasmic reticulum and vacuole.

Increase in cytosolic calcium causes opening of calcium-activated anion channels on the plasma membrane. The opening of anion channels permits large quantities chloride and malate ion to escape from the cells, moving down their electrochemical gradients. The outward flow of negatively charged chloride and malate ions depolarizes the membrane,

triggering the opening of the voltage-gated K^+ efflux channels and closing of voltage-gated K^+ influx channels. ABA also inhibits the activity of plasma membrane H^+ ATP ase, resulting in additional membrane depolarization. The sustained large efflux of both anions and potassium ions from guard cells contributes to loss of guard cell turgor, which leads to stomatal closing (Pei et al. 2000). The effect of ABA on stomatal apertures under red and blue light varies. Increasing ABA concentration inhibits blue light –stimulated stomatal opening in a concentration dependant fashion, but there is no effect on red light stimulated opening. These contrasting responses to blue and red light can be explained by the effect of ABA on guard cell osmoregulation. ABA concentration has been shown to inhibit proton pumping and potassium uptake, which are central to blue light-stimulated opening. Red light, on the other hand, stimulates guard cell photosynthesis and sucrose accumulation and this osmoregulatory pathway appears to be insensitive to ABA.

5.2 Root and shoot growth

ABA has different effect on root and shoot growth, and the effects are strongly dependent on water status of the plant. Under low water potential, when ABA levels are high, the endogenous hormone exerts a strong positive effect on root growth by suppressing ethylene production, and a negative effect on shoot growth (Watts et al. 1981). Endogenous ABA act as a signal to reduce shoot growth only under water stress conditions.

5.3 Dormancy and germination

ABA is required for the development of desiccation tolerance in the developing embryo, the synthesis of storage proteins and the acquisition of dormancy. The high levels of ABA in maturing seeds inhibit germination.

Many types of dormant seeds germinate when ABA is removed or inactivated. Often, the ratio of ABA to gibberllins determines whether the seed remains dormant or germinates. ABA inhibits the GA-dependant hydrolytic enzyme synthesis that is essential for the breakdown of storage reserves in seeds. Although less is known about the role of ABA in bud dormancy, ABA is one of the inhibitors that accumulate in dormant buds (Yan and Chen 2017).

5.4 Vivipary

ABA-deficient embryos may exhibit precocious germination and vivipary. Vivipary is the germination of mature seed within the fruit on maternal plant prior to dispersal. It is rare in angiosperms and is largely restricted to mangroves where seeds germinate while attached to the mother plants and seedlings are shed, stick in to the mud below, and continue to grow (Farnsworth and Farrant 1998). The phenomenon of seedling formation without completing normal embryonic development is called precocious germination. Inactivated ABA or low levels of ABA can lead to precocious germination and vivipary.

5.5 Fruit growth and ripening

The role of ABA in fruit growth and ripening has been described in several studies. ABA seems to help in fruit ripening of tomato where high ABA where high ABA concentrations coincided with stoppage of fruit growth and initiation of coloring (Mou et al. 2016). Some workers believe that ABA might be inducing ethylene production or vice versa. Citrus fruits treated with ethylene had high amount of ABA. Similarly unripe tomato fruits sprayed with ABA underwent quick ripening. Possibly ABA synthesis

takes place in the pericarp of seeds of the fruit. There are also reports available in other succulent fruits that the ABA level increases during the last phase of development. In some ferns there is high ABA accumulation during spore ripening.

5.6 Senescence

ABA plays a multifaceted role in regulating senescence in plants. Its accumulation often coincides with the onset of senescence. It triggers the expression of genes involved in senescence, leading to the breakdown of Chlorophylls, proteins and other macromolecules in Plants tissues (Gao et al. 2016; Zhao et al. 2016). ABA helps in efficient remobilization of nutrients from senescing tissues to other parts of the plant, ensuring essential elements are redistributed before leaf deterioration. Moreover, ABA interacts with other plant hormones like ethylene and cytokinin to regulate senescence. It often works antagonistically with cytokinin, which delay senescence, while interacting synergistically with ethylene to promote senescence.

5.7 Other ABA responses

Recent findings suggest ABA might play a role in lateral or secondary root development. Although auxin predominantly controls the initiation and growth of lateral roots, ABA can impede lateral root development if applied during early stages before the lateral root meristem organization. Previous studies hinted at ABA's impact on flower formation under specific conditions, but the data lacked clarity, failing to establish a direct relationship between endogenous ABA levels and flowering behavior. However, the idea of ABA's involvement in flowering gained renewed attention when ABA-deficient mutants of Arabidopsis displayed earlier

flowering under conditions that typically delay it in wildtype plants. This observation implies that endogenous ABA might typically restrain or postpone flowering in Arabidopsis. Additionally, the discovery that the FCA gene, known for regulating flowering time, also possesses characteristics of an abscisic acid receptor provides further support for ABA's role in flowering (Rai et al., 2024).

6. Signal transduction

The comprehension of ABA perception and signaling remains intricate despite years of studying its metabolism and physiology. ABA, being a weak acid, is likely present in both protonated and unprotonated forms within the relatively acidic apoplast. It might diffuse across the plasma membrane in its protonated state to interact with intracellular receptors, or in its unprotonated form, it could remain external to the cell and be detected by a site on the plasma membrane. Multiple experiments employing impermeable ABA derivatives or microinjections of ABA into cells suggest the presence of several ABA receptors at various locations. Traditional methods for identifying hormone receptors have been largely ineffective in locating ABA receptors. Recently, anti-idiotypic antibodies, created from antibodies raised against ABA, have been employed. These antibodies mimic ABA's binding characteristics, potentially identifying proteins binding with them as putative ABA receptors. ABAP1, found in the plasma membrane of barley aleurone cells, was identified through this method for its specific and reversible ABA binding in vitro. Following ABAP1's discovery, three other putative ABA receptors have been recognized: CHLH (ABAR), FCA, and GCR2. Despite their in vitro ABA binding abilities, confirming them as true receptors requires demonstrating alterations in ABA functions through loss-of-function

or gain-of-function mutants. The intricate signal chain of ABA effects, upstream and downstream of the hormone, undergoes intensive study (Lim et al., 2022). Understanding the interactions between abiotic signals, receptors, second messengers, and ABA-induced gene transcription poses challenges in creating a definitive scheme. Most of the recent progress has been made through newly discovered ABA-insensitive gene mutations and can be summarized in the following points.

- Events sensing abiotic stress and initiating ABA accumulation remain unknown despite rapid ABA turnover in both stressed and unstressed plants.
- Ca^{2+} plays a significant role in the ABA signal chain, especially in stomatal guard cells, mediating ABA-induced turgor adjustments by activating plasma membrane anion channels.
- The promoter region of certain genes contains an ABA response element (ABRE), where transcription factors (ABFs) regulate ABA-induced genes, aiding the plant in adapting to water stress.
- ABA-insensitive mutants like abi3, abi4, and abi5 hinder seed germination and early seedling development, suggesting ABA's role in seed development requires gene transcription.
- ABA-activated protein kinases positively regulate ABA responses, while protein phosphatases like ABI1 and ABI2 negatively regulate them, highlighting the importance of protein phosphorylation in ABA signaling.

Constructing a comprehensive model of the signaling chains for diverse ABA-mediated responses will likely require time, considering the numerous components yet to be discovered.

7. Conclusion

Abscisic acid (ABA) stands out as a pivotal regulator in the realm of plant growth and development. Despite its misleading name, significance of ABA extends far beyond abscission control, influencing various physiological processes across different organisms, including plants, fungi, bacteria, and metazoans. Originally discovered as a growth inhibitor in dormant potato tubers and ash buds, ABA's chemical structure, biosynthesis pathways, and multifaceted functions have been elucidated over the years. With its unique sesquiterpene structure, ABA exists in two enantiomorphic forms, primarily as S-abscisic acid. Its biosynthesis involves both direct synthesis from mevalonic acid and indirect synthesis from carotenoid oxidation. Functionally, ABA plays a central role in stomatal closure, roots and shoots growth modulation, dormancy induction, and germination regulation. Additionally, ABA influences fruit growth and ripening, senescence, and potentially lateral root development and flowering. Understanding its signal transduction mechanisms remains complex, with ongoing research exploring its perception and signaling pathways. In essence, pervasive presence and diverse roles of ABA underscore its importance as a fundamental regulator in plant physiology, offering potential applications in agriculture and biotechnology. Continued research promises deeper insights into its complexities, paving the way for innovative strategies to harness its beneficial effects in plant science and beyond.

References

Arc E, Sechet J, Corbineau F, Rajjou L, Marion Poll A (2013) ABA crosstalk with ethylene and nitric oxide in seed dormancy and germination. Front Plant Sci 4: 63.

Assmann SM, Jegla T (2016) Guard cell sensory systems: Recent insights on stomatal responses to light, abscisic acid, and CO_2. Curr Opin Plant Biol 33: 157–167.

Bauer H, Ache P, Lautner S, Fromm J, HartungW, Al Rasheid KA, Sonnewald S, Addicott FT, Lyon JL, Ohkuma K, Thiessen WE, Carns HR, Smith OE, Cornforth JW, Milborrow BV, Ryback G, Wareing PF (1968) Abscisic acid: A new name for abscisin II (dormin). Science 159: 1493.

Cornforth JW, Milborrow BV, Ryback G, Wareing PF (1965) Chemistry and physiology of 'Dormins' in sycamore: Identity of sycamore 'Dormin' with abscisin II. Nature 205: 1269–1270.

Eagles CF, Wareing PF (1963) Dormancy regulators in woody plants: Experimental induction of dormancy in *Betula pubescens*. Nature 199: 874–875.

Farnsworth EJ, Farrant JM (1998) Reductions in abscisic acid are linked with viviparous reproduction in mangroves. AJB 85(6): 760–769.

Gao S, Gao J, Zhu X, Song Y, Li Z, Ren G, Zhou X, Kuai B (2016) ABF2, ABF3, and ABF4 promote ABA mediated chlorophyll degradation and leaf senescence by transcriptional activation of chlorophyll catabolic genes and senescence associated genes in *Arabidopsis*. Mol Plant 9: 1272–1285.

Hemberg T (1949 a) Growth inhibiting substances in terminal buds of fraxinus. Physiol Plant 2: 37–44.

Hemberg T (1949 b) Significance of growth inhibiting substances and auxins for the rest period of the potato tuber. Physiol Plant 2: 24–36.

Hirai N, Yoshida R, Todoroki Y, Ohigashi H (2000) Biosynthesis of abscisic acid by the non-mevalonate pathway in plants, and by the mevalonate pathway in fungi. Biosci Biotechnol Biochem 64: 1448–1458.

Hirayama T, Shinozaki K (2007) Perception and transduction of abscisic acid signals: Keys to the function of the versatile plant hormone, ABA. Front Plant Sci 2: 343–351.

Izquierdo Bueno I, Gonzalez Rodriguez VE, Simon A, Dalmais B, Pradier JM, Le Pecheur P, Mercier A, Walker AS, Garrido C, Collado IG, Viaud M (2018) Biosynthesis of abscisic acid in fungi: Identification of a sesquiterpene cyclase as the key enzyme in Botrytis cinerea. Environ Microbiol 20: 2469–2482.

Lim J, Lim CW, Lee SC (2022) Core components of abscisic acid signaling and their post-translational modification. Front Plant Sci 13: 895698.

Martin StPaul N, Delzon S, Cochard H (2017) Plant resistance to drought depends on timely stomatal closure. Ecol Lett 20:1437–1447.

Mou W, Li D, Bu J, Jiang Y, Khan ZU, Luo Z, Mao L, Ying T. (2016). Comprehensive analysis of ABA effects on ethylene biosynthesis and signaling during tomato fruit ripening. PLoS One 11 11(4): e0154072.

Nambara E, Marion Poll A (2005) Abscisic acid biosynthesis and catabolism. Annu Rev Plant Biol 56: 165–185.

Ohkuma K, Lyon JL, Addicott FT, Smith OE (1963) Abscisin II, an abscission accelerating substance from young cotton fruit. Science 142: 1592–1593.

Pei ZM, Murata Y, Benning G, Thomine S, Klusener B, Allen GJ, Grill E, Schroeder JI (2000) Calcium channels activated by hydrogen peroxide mediate abscisic acid signaling in guard cells. Nature 406: 731–734.

Rai GK, Khanday DM, Choudhary SM, Kumar P, Kumari S, Martínez-Andújar C, Martínez-Melgarejo PA, Rai PK, Pérez-Alfocea F (2024) Unlocking nature's stress buster: Abscisic acid's crucial role in defending plants against abiotic stress. Plant Stress 11: 100359.

Ruth Finkelstein (2013) Abscisic acid synthesis and response, The *Arabidopsis* B 11: 234–251.

Sonnewald U, Kneitz S, Lachmann N, Mendel RR, Bittner F, Hetherington AM, Hedrich R (2013) The stomatal response to reduced relative humidity requires guard cell autonomous ABA synthesis. Curr Biol 23: 53–57.

Su J, Zhang M, Zhang L, Sun T, Liu Y, Lukowitz W, Xu J, Zhang S (2017) Regulation of stomatal immunity by interdependent functions of a pathogen responsive MPK3/ MPK6 cascade and abscisic acid. Plant Cell 29: 526–542.

Watts S, Rodriguez JL, Evans SE, Davies WJ (1981) Root and shoot growth of plants treated with Abscisic acid. Ann Bot 47: 595–602.

Takino J, Kozaki T, Sato Y, Liu C, Ozaki T, Minami A, Oikawa H (2018) Unveiling biosynthesis of the phytohormone abscisic acid in fungi: Unprecedented mechanism of core scaffold formation catalyzed by an unusual sesquiterpene synthase. J Am Chem Soc 140: 12392–12395.

Yan A, Chen Z (2017) The pivotal role of abscisic acid signaling during transition from seed maturation to germination. Plant Cell Rep 36: 689–703.

Zhao Y, Chan Z, Gao J, Xing L, Cao M, Yu C, Hu Y, You J, Shi H, Zhu Y, Gong Y, Mu Z, Wang H, Deng X, Wang P, Bressan RA, Zhu JK (2016) ABA receptor PYL9 promotes drought resistance and leaf senescence. Proc Natl Acad Sci USA 113: 1949–1954.

The Multifaceted Role of Ethylene in Plant Growth

Hibamol P. K.

PG Department of Botany, Korambayil Ahamed Haji Memorial Unity Women's College, Manjeri, Malappuram, 676122, Kerala, India.

Email: hibam362@gmail.com

Abstract

Ethylene, a simple gaseous plant hormone, plays a pivotal role in regulating various physiological processes throughout the plant life cycle. Ethylene influences diverse processes including seed germination, seedling growth, leaf and flower senescence, fruit ripening, and response to biotic and abiotic stresses. The biosynthesis of ethylene involves a well-coordinated pathway primarily initiated by the conversion of methionine to S-adenosyl-L-methionine (SAM), which is subsequently converted to 1-aminocyclopropane-1-carboxylic acid (ACC). The final step, catalyzed by the enzyme ACC oxidase, leads to the production of ethylene. Ethylene perception and signaling occur through a receptor-mediated pathway involving ethylene receptors, constitutive triple response 1 (CTR1), and downstream transcription factors such as Ethylene Insensitive 3 (EIN3) and Ethylene Response Factors (ERFs). Ethylene regulates growth processes by modulating cell expansion, cell division, and differentiation. It also plays a crucial role in coordinating responses to various stresses including drought, salinity, pathogens, and mechanical injury. Ethylene's involvement in senescence regulation and fruit ripening is well documented, where it acts as a key regulator of senescence-related genes and enzymes responsible for fruit

softening and flavor development. Moreover, ethylene interacts with other phytohormones such as auxins, cytokinins, gibberellins, and abscisic acid, orchestrating complex crosstalk to fine-tune plant growth and development. Additionally, recent advancements in molecular genetics and omics technologies have provided deeper insights into the intricate regulatory networks underlying ethylene signaling and its cross-interactions with other hormones. Understanding the precise mechanisms by which ethylene influences plant physiology is crucial for agricultural applications, including the manipulation of ethylene signaling pathways to enhance crop yield, improve stress tolerance, and optimize fruit quality. This review underscores the significance of ethylene as a central player in the intricate network of plant hormone signaling highlighting avenues for future research and potential biotechnological interventions in agriculture.

Keywords: Bioengineering, Hormone receptor, Phytohormone, Signal transduction.

1. Introduction

Ethylene (C_2H_4) is a simple gaseous hydrocarbon that has profound effects upon plant growth and development. Besides being associated with ripening, ethylene plays a role throughout the entire life of the plant. Ethylene is a regulator of seed germination, seedling growth, leaf and petal abscission, organ senescence, stress responses, and pathogen responses. The production of ethylene is tightly regulated by internal signals during development and in response to environmental stimuli from biotic (e.g., pathogen attack) and abiotic stresses, such as wounding, hypoxia, ozone, chilling, or freezing. To understand the roles of ethylene in plant functions, it is important to know how this gaseous hormone is synthesized, how its

production is regulated, and how the signal is transduced. Ethylene was one of the first plant hormones discovered (Bleecker and Kende 2000). In the nineteenth and early twentieth centuries, illuminating gas produced from coal was used for lighting. Leaks from pipelines carrying illuminating gas resulted in premature senescence and abscission in nearby vegetation, sometimes seriously damaging trees and greenhouse plants. Dimitry Neljubov identified ethylene as the "active" component in illuminating gas and published his results in 1901. In the 1930s, plants were demonstrated to produce ethylene themselves, thereby establishing ethylene as an endogenous regulator of plant growth and development.

2. Structure of ethylene

Ethylene is a hydrocarbon which has the formula C_2H_4 or $H_2C{=}CH_2$. It is a colourless, flammable gas with a faint "sweet and musky" odour when pure. It is the simplest alkene (a hydrocarbon with carbon-carbon double bond) (Fig. 1).

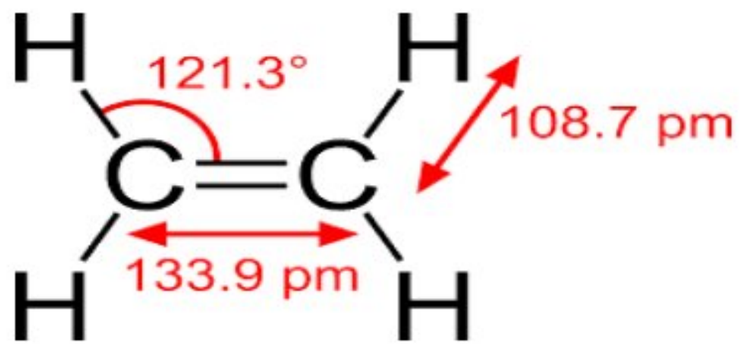

Figure 1. Structure of Ethylene

3. Discovery of ethylene

The discovery of ethylene as a plant hormone indeed stemmed from the unintended presence of ethylene in the environment. In the 1800s, when illuminating gas (coal gas) was commonly used for lighting, its leakage from gas lines caused noticeable damage to plants, such as defoliation of trees

around streetlamps. Dimitry Neljubow, towards the end of the 19th century, observed peculiar growth in etiolated pea seedlings in his laboratory, which was attributed to leaking illuminating gas. He noted that the seedlings exhibited shortened and thickened epicotyls with horizontal bending. Neljubow identified ethylene as the biologically active component of illuminating gas. This observation sparked further research into the effects of ethylene on plants. In 1934, Richard Gane discovered that plants themselves synthesize ethylene. This finding was significant as it correlated ethylene biosynthesis with biological activity, which was a crucial step in convincing researchers that a gas could function as a plant hormone. Indeed, ethylene became the first identified gaseous signaling molecule in any organism, paving the way for extensive studies on its wide-ranging effects on plant growth, development, and responses to environmental stimuli.

4. Biosynthesis of ethylene

The elucidation of the ethylene biosynthetic pathway, primarily through the groundbreaking work of Yang and co-workers, has provided crucial insights into the molecular mechanisms governing this essential plant hormone. Derived from the amino acid methionine, ethylene production initiates with the conversion of methionine to S-adenyl-methionine (AdoMet) catalyzed by AdoMet synthetase. The pivotal and often rate-limiting step in ethylene biosynthesis is carried out by ACC synthase, which transforms AdoMet into 1-aminocyclopropane-1-carboxylic acid (ACC). Arabidopsis, a model plant, possesses thirteen ACC synthase genes, with some potentially non-functional. Notably, the expression of ACS2, a key player in lateral root formation, is high in young tissues but diminishes with tissue maturation (Bleecker and Kende 2000).

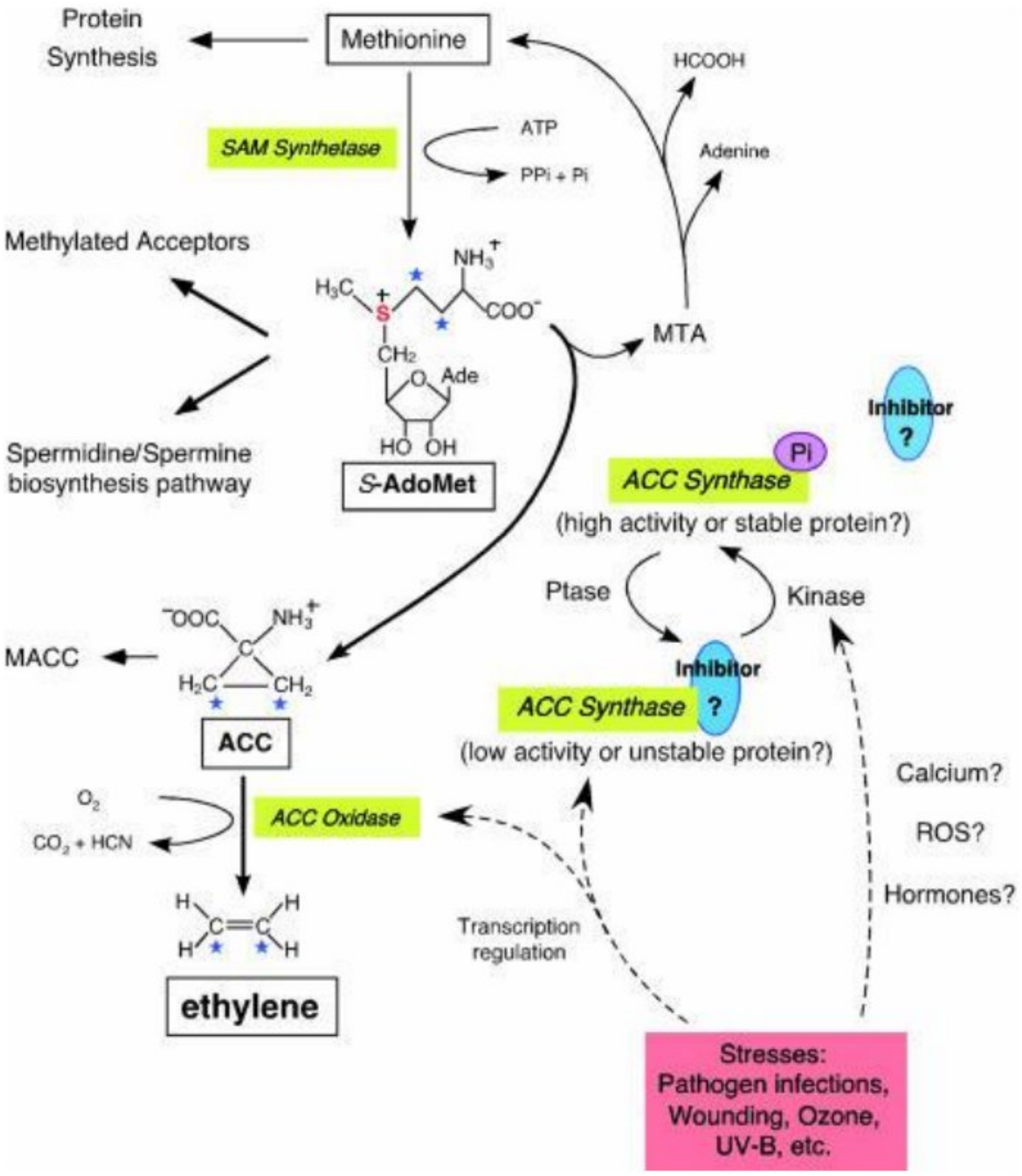

Figure 2. Biosynthetic pathway and regulation of ethylene. The formation of S-AdoMet is catalyzed by SAM synthetase from the methionine at the expense of one molecule of ATP per molecule of S-AdoMet synthesized. S-AdoMet is the methyl group donor for many cellular molecules (Methylated Acceptors), including nucleic acids, proteins, and lipids. In addition, S-AdoMet is the precursor of the polyamine synthesis pathway (Spermidine/Spermine biosynthesis pathway). ACC is the immediate precursor of ethylene. The rate-limiting step of ethylene synthesis is the conversion of S-AdoMet to ACC by ACC synthase under most conditions. MTA is the by-product generated along with ACC production by ACC synthase. Recycling of MTA back to methionine conserves the methylthio

group and is able to maintain a constant concentration of cellular methionine even when ethylene is rapidly synthesized. Malonylation of ACC to malonyl-ACC (MACC) deprives the ACC pool and reduces the ethylene production. ACC oxidase catalyses the final step of ethylene synthesis using ACC as substrate and generates carbon dioxide and cyanide. Transcriptional regulation of both ACC synthase and ACC oxidase is indicated by dashed arrows. Reversible phosphorylation of ACC synthase is hypothesized and may be induced by unknown phosphatases (Ptase) and kinases, the latter presumably activated by stresses. Both native and phosphorylated form (ACC synthase-Pi) of ACC synthase is functional, although the native ACC synthase may be less stable or active in vivo. A hypothetical inhibitor is associated with ACC synthase at the carboxyl end and may be dissociated from the enzyme if it is modified by phosphorylation at the vicinity. (1-aminocyclopropane-1-carboxylic acid (ACC), S-adenyl-methionine (AdoMet), 5'- Methylthioadenosine (MTA) (Wang et al. 2002).

The final conversion of ACC to ethylene is facilitated by ACC oxidase (ACO). A multigene family in Arabidopsis, ACO genes, including AtACO2, exhibit differential expression patterns, with AtACO2 implicated in apical hook development. Ethylene production is modulated by a myriad of factors, such as auxin, cytokinin, brassinosteroids, and environmental stimuli, influencing the steady-state levels of ACS mRNA. Of particular interest is the post-transcriptional regulation of ACS5, a major target for cytokinin modulation, demonstrated by mutations like eto2, affecting the C-terminus of ACS5. Additionally, other mutations, like eto1 and eto3, emphasize the importance of post-transcriptional regulation, with the ETO1 gene playing a role in the post-translational control of ACS5 function. This intricate network of regulatory mechanisms underscores the dynamic nature

of ethylene biosynthesis in plants, pivotal for various developmental processes and responses to environmental cues (Fig. 2).

5. Biosignaling

Major breakthroughs in understanding the ethylene signaling pathway came from molecular genetic dissection of the pathway in the flowering plant Arabidopsis thaliana, initiated by the isolation of Arabidopsis ethylene response mutants. Mutants were isolated in the late 1980s concomitant with the development of *Arabidopsis* as a genetic plant model, using a powerful genetic screen based on Neljubow's observation in etiolated pea seedlings. In response to ethylene, etiolated Arabidopsis seedlings exhibit a short and thick hypocotyl, an exaggerated apical hook and a short root. This phenotype, coined the "triple response", is easily induced in the laboratory and is highly specific to ethylene. Cloning of the corresponding genes using map-based methods, such as chromosome walking, led to the identification of several key components of the pathway, including the first known plant hormone receptor, ETR1. Today, all of the central elements in ethylene signaling have been identified in Arabidopsis, and key mechanistic aspects of the pathway have been elucidated using a combination of genetics, molecular biology, cell biology and biochemistry. Studies in other plant species, particularly tomato, have further elaborated on and supported these findings. The ethylene signaling pathway is highly conserved in plants and dates back to an algal ancestor prior to the colonization of land more than 450 million years ago.

5.1 Ethylene signaling pathway

Ethylene signaling involves a unique pathway that consists of the following main steps: (i) ethylene is perceived by an ethylene receptor complex at the endoplasmic reticulum (ER) membrane; (ii) ethylene de tection triggers cleavage of a key protein in the complex, ETHYLENE-INSENSITIVE2 (EIN2); (iii) the cleaved soluble portion of EIN2 is involved in repressing the translation of two regulatory F-box proteins, which would otherwise target two master transcription factors for degradation by the 26S proteasome; and (iv) rapid stabilization of the two transcription factors results in the regulation of gene expression. The pathway relies heavily on negative regulation and post-translational controls. For example, as explained below, the ethylene receptors repress responses when no ethylene is detected (as opposed to activating responses when ethylene is detected), and the repression of ethylene responses involves protein phosphorylation and protein turnover (Chang 2016).

5.2 Ethylene receptor

The ethylene receptor is unexpectedly related to the histidine protein kinase receptors of the two-component signaling system, which is prevalent in prokaryotes but very rare in eukaryotes. It is believed that plants most likely acquired the ethylene receptor gene from an ancient endosymbiotic cyanobacterium that became the chloroplast. Plants have a small family of ethylene receptors (e.g., *Arabidopsis* has five ethylene receptors and tomato has six) that have overlapping and distinct functions. As in typical prokaryotic two component receptors, the ethylene receptor has an N terminal ligand-binding domain followed by a GAF domain and a histidine protein kinase domain. Some iso forms also have a C-terminal receiver

domain, which is the second element of the two-component system. In the ethylene receptors, the ethylene-binding domain lies within the ER membrane while the GAF, histidine kinase and receiver domains are in the cytoplasm. It is un clear why the ethylene receptors reside at the ER membrane, but given the diffusion of ethylene across membranes, there is no obligation for the receptor to be at the cell surface. Ethylene is more soluble in hydrophobic environments, consistent with the location of the ethylene-binding pocket within the membrane. The ethylene receptors form disulfide-linked dimers, and each dimer is capable of binding a single ethylene molecule with the help of a copper ion cofactor The dimers reside in clusters at the ER membrane where they interact with downstream proteins in the pathway. The GAF domain, usually known for binding small molecules, facilitates protein–protein interactions between ethylene receptor monomers as well as between isomers (Rüffer et al. 2024).

5.3 How do the ethylene receptors signal?

Ethylene responses are repressed by ethylene receptor signaling. This repression occurs in the absence of ethylene binding and is achieved through receptor activation of CONSTITUTIVE RESPONSE1 (CTR1), a serine/threonine protein kinase that has sequence similarity to the Raf protein kinase family. CTR1 kinase activity negatively regulates the pathway (i.e., prevents downstream signaling). When ethylene binds to the receptors, ethyl ene receptor signaling ceases. Consequently, CTR1 is no longer activated and downstream ethylene signal ing can proceed. This model is supported by the fact that null mutations in multiple ethylene receptor genes display constitutive ethylene responses similar to ctr1 loss-of-function mutants, whereas dominant, gain-of-function receptor mutations confer ethylene in sensitivity (Binder 2020).

5.4 The biochemical mechanism of ethylene receptor signaling

This is still unresolved. In the canonical two-component system, binding of the ligand either stimulates or inhibits, autophosphorylation of a conserved histidine residue followed by transfer of the phosphate to a conserved aspartate in the receiver domain. Curiously, histidine kinase activity does not appear to play a major role in ethylene receptor signaling. Although the ethylene receptors display histidine and/or serine/threo nine kinase activity in vitro, neither activity has been de finitively associated with ethylene signaling. In addition, despite hints of two-component signaling elements acting downstream of the receptors, there is strong evidence in dicating that this is not the primary mode of ethylene sig naling. Instead, the ethylene receptors physically associate with and signal to CTR1. The receptors also show interaction with the phosphorylation substrate of CTR1, ETHYLENE-INSENSITIVE2 (EIN2).

Although genetic evidence indicates that ethylene binding inhibits receptor signaling, there is no clear answer to the basic question: Does the binding of ethylene stimulate or inhibit biochemical activity in the receptor? There are data to support each possibility. Although it might be counter-intuitive, a formal possibility is that CTR1 activation occurs by a passive (e.g., steric-based) signaling mechanism that is alleviated when ethylene re ceptor activity is triggered by the binding of ethylene (Ju and Chang 2015; Chang 2016).

5.5 Downstream of the receptors in the ethylene signaling pathway

Ethylene signaling downstream of CTR1 hinges on the phosphorylation status of EIN2, an enigmatic central regulator of the

ethylene-signaling pathway [23]. EIN2 is tethered to the ER membrane by its N-terminal domain, which has sequence similarity to the widely conserved NRAMP metal ion transporters, but the biochemical function of this domain and its role in ethylene sig naling have yet to be determined. The C-terminal portion (C-END) of EIN2 consists of a novel plant-specific domain that is cytosolic, and expression of this domain alone is sufficient for the activation of ethylene responses.

In the absence of ethylene, the CTR1 kinase phosphorylates the EIN2 C-END, thereby preventing the C-END from signaling. When the receptors detect ethylene, CTR1 is inactivated, and consequently the unphosphorylated EIN2 C-END is proteolytically released from the ER-anchored NRAMP domain. The cleaved C-END then represses the translation of two F-box proteins, EIN3-BINDING F-BOX1 and 2 (EBF1/2), by binding to the 3' untranslated regions of EBF1/2 mRNA. This repression, which occurs within discrete cytoplasmic domains (known as P-bodies) where mRNA fates are decided, is crucial in ethylene signaling, because in the nucleus, the EBF1/2 proteins control the proteolytic degradation of two master transcription factors, EIN3/EIL1, which are required for essentially all known ethylene responses.

In the absence of ethylene, EBF1/2 target EIN3/EIL1 for ubiquitylation and degradation, in an SCFEBF1/EBF2 ubiquitin-ligating complex; this is yet another example of negative regulation in the pathway. When ethylene is perceived, EIN2 represses translation of EBF1/2, thereby permitting the EIN3/EIL1 transcription factors to quickly accumulate in the nucleus, leading to rapid re sponses to ethylene. There is also evidence that the cleaved EIN2 C-END must enter the nucleus in order to activate

downstream ethylene signaling, but the exact function of the C-END in the nucleus is unknown.

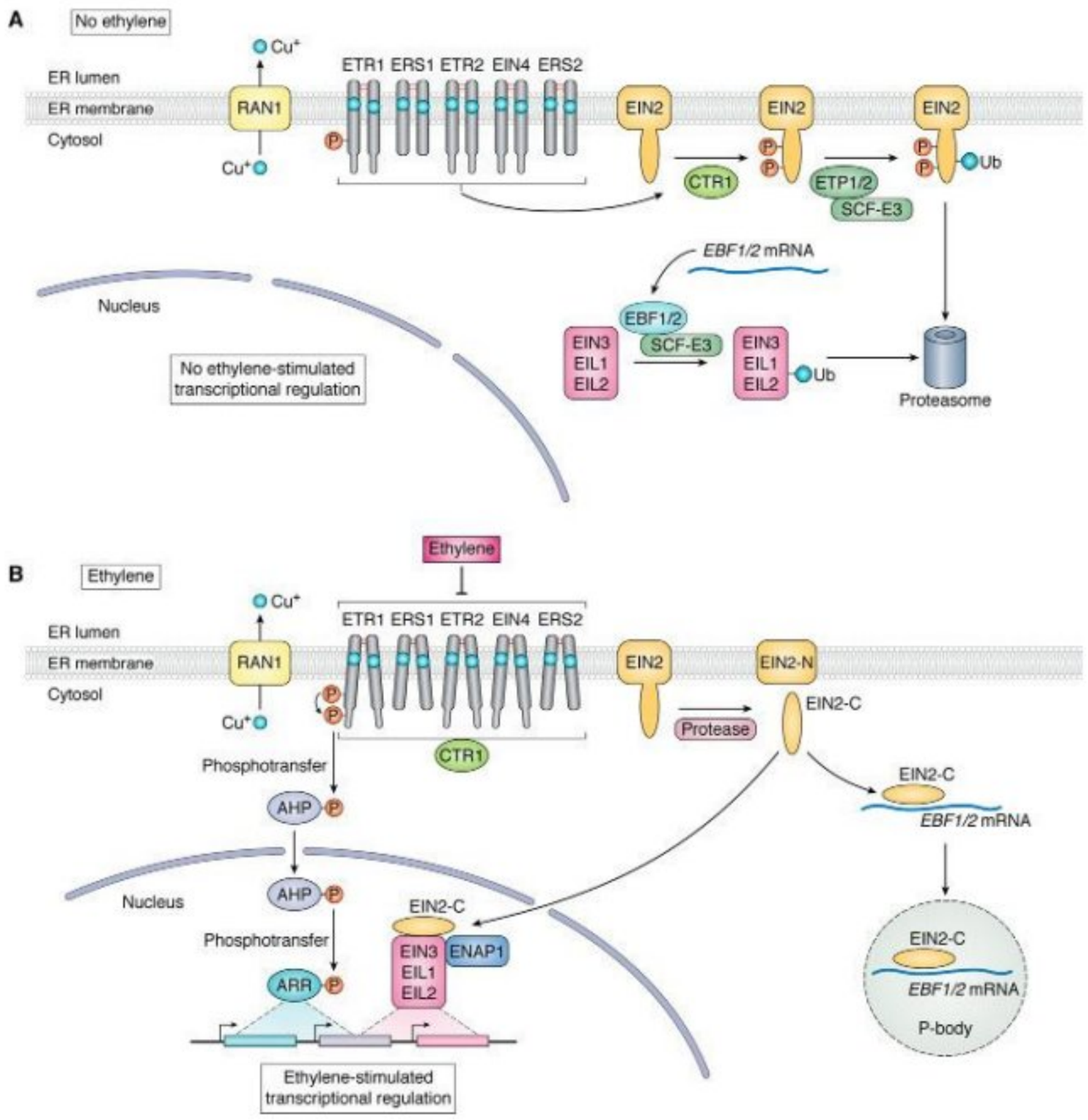

Figure 3. Model for ethylene signaling. RAN1 is a copper transporter that delivers copper to the lumen of the ER, where it is required for the biogenesis of the receptors and is used as a cofactor by the receptors to bind ethylene. A, in the absence of ethylene, the receptors signal to CTR1, which phosphorylates EIN2. This results in the ubiquitination of EIN2 by an SCF E3 containing the ETP1/2 F-box proteins, leading to EIN2 degradation by the proteasome. Because EIN2 levels are low, an SCF-E3 containing the EBF1/2 F-box proteins ubiquitinates EIN3 and EIL1, leading to their degradation by the proteasome and preventing them from affecting transcription in the nucleus. B, in the presence ethylene, the receptors bind

ethylene via a copper cofactor. The binding of ethylene is modeled to cause a conformational change that either reduces CTR1 kinase activity or, as shown, results in CTR1 being sequestered by the receptors so that CTR1 can no longer phosphorylate EIN2. The reduction in EIN2 phosphorylation results in less EIN2 ubiquitination and an increase in EIN2 levels. An unknown protease cleaves EIN2, releasing the C-terminal end (EIN2-C) from the N-terminal end (EIN2-N). One fate of EIN2-C is to bind the RNAs for EBF1 and EBF2 and become sequestered in processing bodies (P-bodies). The reduction of EBF1/2 results in less ubiquitination of EIN3 and EIL1, causing higher EIN3/EIL1 levels. The other fate of EIN2-C is to translocate to the nucleus, where it increases the transcriptional activity of EIN3/EIL1 via ENAP1. This leads to numerous transcriptional changes. In parallel with this pathway, phosphoryl transfer from a conserved histidine in the ETR1 DHp domain to an aspartate in the receiver domain occurs. This is followed by phosphoryl transfer from this residue to AHPs and finally ARRs resulting in transcriptional changes (Wang et al. 2002).

6. Functions of ethylene

Ethylene is a hydrocarbon gas that serves various important functions in plant physiology, industry, and chemical synthesis. Here are some of its primary functions:

6.1 Leaf growth and development

Ethylene, a key phytohormone, has been demonstrated to play a pivotal role in the growth and development of leaf through physiological

studies utilizing ethylene inhibitors and genetic investigations involving ethylene-insensitive mutants or transgenic plants lacking essential ethylene biosynthesis enzymes. The Arabidopsis ETHYLENE RESPONSE FACTOR5 (ERF5) and ERF6 have been identified as contributors to improved leaf growth under environmental challenges. However, the response to ethylene is not uniform, varying with concentration and plant species. Studies on Poa species and mustard indicate differential responses, with slower-growing species exhibiting greater inhibition in leaf elongation at higher concentrations but promoting leaf elongation at lower concentrations. Ethylene-insensitive genotypes of various plants, including Arabidopsis, tobacco, and petunia, displayed no increase in total leaf area compared to ethylene-sensitive controls. Interestingly, contrasting findings in maize, where the rate of ethylene evolution did not correlate with leaf elongation variability, highlight the complexity of ethylene's effects. Additionally, reports of ethylene-induced reductions in leaf growth in pea plants with rhizobacteria and in lettuce grown in stressful ethylene-rich environments emphasize the environmental sensitivity of these processes. The impact on leaf area in lettuce was linked to ethylene's indirect effects on leaf epinasty, light capture, and CO2 assimilation, further underscoring the multifaceted nature of ethylene's role. Furthermore, the involvement of reactive oxygen species (ROS) and nitric oxide (NO), potentially influenced by ethylene, adds another layer to the intricate regulation of leaf expansion (Schaller 2012; Dubois et al. 2018).

6.2 Leaf senescence

Ethylene plays a crucial role in the intricate regulation of leaf senescence, with significant impacts on various physiological and molecular processes. Ethylene stands out as one of the foremost hormones involved in

the regulation of leaf senescence. Its ability to initiate the senescence process is particularly notable in sensitive plant species. Ethylene biosynthesis exhibits a dynamic pattern, peaking during the initial stages of leaf formation, declining until maturity, and surging again during the onset of senescence. The ACC (1-aminocyclopropane-1-carboxylic acid) content mirrors this pattern, rising exclusively in senescing leaves. At the molecular level, ethylene's involvement is orchestrated by gene expression, where different gene family members encode enzymes of ethylene biosynthesis, activated in a timely manner during leaf development (Pandey et al. 2000).

Leaf senescence, a process activated at the mature stage of leaf development, unfolds through three distinct stages: initiation, organization of degradation, and death processes. Visible symptoms of leaf senescence include chlorophyll degradation and leaf abscission. The yellowing of leaves, resulting from chlorophyll breakdown catalyzed by chlorophyllase, is a hallmark of senescence. Ethylene exposure exacerbates chlorophyll loss, leading to visible senescence symptoms such as malformed leaves and epinasty. Additionally, ethylene induces abscission and necrosis, marked by structural changes in cells within the abscission zone. The impact of ethylene on leaf senescence varies with leaf age and treatment duration. In various plants, including cut flowers like stock and chrysanthemum, ethylene exposure accelerates chlorophyll degradation. The age of the leaves and the duration of ethylene treatment significantly influence the observed effects. For instance, tobacco leaves exhibit increased chlorophyll degradation after 24 hours of ethylene treatment, with implications for respiration patterns. Ethylene-induced effects also extend to other aspects of leaf senescence, such as the reduction of leaf shelf life in rocket salad leaves. The intricate involvement of ethylene in leaf senescence underscores its multifaceted

impact on plant physiology and molecular processes, demonstrating its role in orchestrating the orderly dismantling of cells and facilitating nutrient recycling from senescing leaves to other plant organs (Iqbal et al. 2017).

6.3 Floral development

The floral transition, a pivotal stage in the plant life cycle signaling conditions conducive to reproductive success, is intricately regulated by ethylene, a plant hormone. Studies in Arabidopsis and rice, examining ethylene-related mutants and their responses compared to wild-type plants, reveal the complex role of ethylene in the transition from vegetative to reproductive growth. Ethylene-overproducing mutants exhibit early flowering, while ethylene-insensitive mutants display delayed flowering. Contrasting roles of ethylene are observed in rice. Inhibitors of ethylene biosynthesis delay flowering, as seen in pineapple. Ethylene receptors are implicated in reproductive organ development, with tissue-specific expression observed in China rose and pineapple. Flower development involves the sequential regulation of ethylene biosynthesis genes, indicating a link to specific flower tissues. A potential genetic network emerges, with ethylene influencing flower development and interacting with homeotic genes. The intricate interplay of ethylene in flower specification and the regulation of floral organ identity genes is evident in various plant species, emphasizing its fundamental role in flower development (Achard et al., 2007).

6.4 Flower senescence

Among plant hormones, ethylene plays a key role in flower senescence and aging. Ethylene's involvement in flower petals includes the

regulation of water channel proteins (aquaporins), impacting cell expansion and transmembrane water transport. The role of aquaporins in flower development is highlighted by the significant transcriptional regulation of aquaporin-encoding genes throughout different flower developmental stages. While a large number of flowers are affected by ethylene, sensitivity varies among species and cultivars. Pollination-induced ethylene production triggers senescence in ethylene-sensitive species, leading to a cascade of cellular events and eventual wilting. Pharmacological treatments targeting the ethylene signaling pathway, such as AVG, AOA, STS, and 1-MCP, demonstrate the intricate network of interactions involved in flower senescence. Exogenous application of ethylene or its precursor accelerates corolla senescence, while inhibitors of ethylene biosynthesis delay senescence.

The synthesis of ethylene involves enzymes like ACC synthase (ACS) and ACC oxidase (ACO), with their increased expression during flower senescence. Antisense technology targeting ACS and ACO genes has been successful in delaying floral senescence in various ornamental species. The rate-limiting enzyme ACS may also be regulated post-transcriptionally, as seen in Arabidopsis and petunia. Positive feedback regulation in China rose flowers involves an increase in ethylene production through the activation of ACS and/or ACO during senescence. Global transcriptome profiling of China rose indicates that senescence is driven by the upregulation of the ethylene biosynthetic pathway and differential regulation of ethylene response factors (ERFs) among flower tissues during aging (Haq et al. 2023).

6.5 Fruit ripening

The ripening of fruits involves a complex coordination of biochemical and developmental pathways, primarily regulated by the plant hormone ethylene. Ethylene influences various aspects of ripening, including color changes, texture, nutritional quality, and aroma. In climacteric fruits, the ripening process is tightly regulated by ethylene, influencing firmness and color changes. Ethylene is closely associated with the biosynthesis of volatile organic compounds (VOCs) in ripe fruits, enhancing their attraction to frugivores. Inhibition of ethylene biosynthesis results in reduced VOC production, impacting the aroma of fruits. Transgenic fruits expressing antisense genes for ACS or ACO, enzymes involved in ethylene biosynthesis, exhibit lower VOC levels. Exogenous application of ethylene can reverse the reduction in VOCs, indicating the inhibitory role of ethylene in volatile biosynthesis. Pharmacological treatments with 1-MCP or AVG demonstrate that ethylene regulates VOC biosynthesis both directly and indirectly through the ethylene perception pathway (Liu et al. 2015; Iqbal et al. 2017).

The relationship between fruit ripening and ethylene/respiration patterns allows the classification of fruits into climacteric or non-climacteric categories. In climacteric fruits, ethylene production increases with a peak corresponding to the respiration pattern, while in non-climacteric fruits, ethylene declines during ripening and senescence. Tomato has served as a model plant for studying the role of ethylene in fruit ripening. Two systems, system 1 and system 2, explain the auto-inhibitory and auto-stimulatory effects of ethylene during vegetative growth and ripening, respectively. Ethylene-regulated genes, ACS6 and ACS1, control ethylene biosynthesis during system 1. Inhibiting ethylene biosynthesis or action delays the increase in ethylene, commonly employed to extend the shelf life of

climacteric fruits. Ethylene also influences the expression of genes involved in polygalacturonase activity, pectin methylesterase, and phytoene synthase during fruit ripening, affecting cell wall structure, pigmentation, and texture. Mutations in ethylene receptors, such as Never-ripe (Nr), impact fruit development, morphology, and the expression of numerous genes during maturation. The E8 gene in tomatoes negatively regulates ethylene biosynthesis, and its repression results in increased ethylene evolution but delayed ripening (Fan et al. 2022).

6.6 Fruit senescence

The softening of fruits, a crucial quality parameter, results from the degradation of the cell wall facilitated by a set of enzymes, including pectin methyl esterases, polygalacturonase, cellulase, galactosidases, pectate lyase (PL), xyloglucan transglucosylase/hydrolases, and expansins. These enzymes, often encoded by multigene families, exhibit spatial-temporal regulation during fruit development and senescence. Ethylene plays a pivotal role in orchestrating the activation of these genes and enzymes throughout the ripening and senescence processes. Expansins, proteins involved in cell matrix enlargement during cell wall growth and disruption, are particularly influenced by ethylene and pH dependence. The transcription of these enzymes is governed by gene families, and specific isoforms are activated at different developmental stages.

Among these enzymes, pectin methyl esterases (PME) catalyze the de-esterification of pectin, a crucial step before fruit ripening, allowing polygalacturonase action. PME is stimulated by ethylene, and its activity is inhibited by ethylene inhibitors like 1-MCP. Exo- and endopolygalacturonase, involved in galacturonic acid depolymerization, are

activated post PME action and induced by ethylene. β-galactosidase, responsible for breaking bonds in the cell wall, contributes to fruit softening, and its activity is influenced by ethylene. Transgenic tomatoes with antisense β-galactosidase exhibit higher firmness, and inhibiting ethylene action in avocados reduces β-galactosidase activity. Pectate lyase (PL) is involved in α-d-galacturonic acid breakdown, and its activity increases with ethylene treatment in bananas and decreases with the use of 1-MCP in mangoes. The sequential activation of these cell wall-degrading enzymes during ripening and senescence is intricately regulated by ethylene at both transcriptional and post-transcriptional levels (Shin et al. 2021).

6.7 Fruit de-greening

Ethylene is sometimes used to accelerate the process of de-greening in certain fruits, making them more visually appealing for consumers. It's important to note that while ethylene has several beneficial applications, it also poses challenges in industries such as fruit storage and transportation, where controlling ethylene levels is crucial to extending the shelf life of produce. Additionally, the environmental impact of plastic production using ethylene has led to increased interest in sustainable alternatives (Tucker et al. 2017).

7. Conclusion

Ethylene, a crucial phytohormone, plays a central role in plant growth and development by triggering signaling pathways and interacting with other phytohormones. The crosstalk between ethylene and other hormones influences various processes, including the transition from vegetative to reproductive stages and senescence. Manipulating hormone content through

molecular techniques offers a promising strategy to elicit specific plant responses. Studies on molecular changes in plant tissues following combined treatments of ethylene with other hormones are essential for a comprehensive understanding. Examining different organs and developmental stages can reveal insights into the intricate network affecting agronomic traits such as yield, longevity, and morphology. Discovering new relationships among ethylene and other hormones has the potential to support cell division, enhance crop yield, delay aging, prolong flower shelf-life, and maintain climacteric fruit quality. Additionally, investigating the equilibrium between ethylene biosynthesis and perception under various stress conditions is crucial for understanding crop adaptability. The interplay of ethylene and other hormones at the post-translation level should be explored to gain deeper insights into plant performance.

References

Haq AU, Farooq S, Lone ML, Parveen S, Altaf F, Tahir I (2023) Flower senescence coordinated by ethylene: An update and future scope on postharvest biology in the "Buttercup" family. J Plant Growth Regul 43: 402–422.

Achard P, Baghour M, Chapple A, Hedden P, Van Der Straeten D, Genschik P, Moritz T, Harberd NP (2007) The plant stress hormone ethylene controls floral transition via DELLA-dependent regulation of floral meristem-identity genes. Proc Natl Acad Sci U S A 104(15): 6484–6489.

Dubois M, Van den Broeck L, Inzé D (2018) The pivotal role of ethylene in plant growth. Trends Plant Sci 23(4): 311–323.

Schaller GE (2012) Ethylene and the regulation of plant development. BMC Biol 10: 1–3.

Ju C, Chang C (2015) Mechanistic insights in ethylene perception and signal transduction. Plant Physiol 169(1): 85–95.

Binder BM (2020) Ethylene signaling in plants. J Biol Chem 295(22): 7710–7725.

Rüffer B, Thielmann Y, Lemke M, Minges A, Groth G (2024) Crystallization of ethylene plant hormone receptor–screening for structure. Biomolecules 14(3): 375.

Bleecker AB, Kende H (2000) Ethylene: a gaseous signal molecule in plants. Annu Rev Cell Dev 16(1): 1–18.

Pandey S, Ranade SA, Nagar PK, Kumar N (2000) Role of polyamines and ethylene as modulators of plant senescence. J Biosci 25: 291–299.

Tucker G, Yin X, Zhang A, Wang M, Zhu Q, Liu X, Xie X, Chen K, Grierson D (2017) Ethylene and fruit softening. Food Qual Saf 1(4): 253–267.

Shin Y, Chane A, Jung M, Lee Y (2021) Recent advances in understanding the roles of pectin as an active participant in plant signaling networks. Plants 10(8): 1712.

Fan D, Wang W, Hao Q, Jia W (2022) Do non-climacteric fruits share a common ripening mechanism of hormonal regulation? Front Plant Sci 13: 923484.

Liu M, Pirrello J, Chervin C, Roustan JP, Bouzayen M (2015) Ethylene control of fruit ripening: revisiting the complex network of transcriptional regulation. Plant Physiol 169(4): 2380–2390.

Iqbal N, Khan NA, Ferrante A, Trivellini A, Francini A, Khan MIR (2017) Ethylene role in plant growth, development and senescence: Interaction with other phytohormones. Front Plant Sci 8: 475.

Wang KL, Li H, Ecker JR (2002) Ethylene biosynthesis and signaling networks. Plant Cell 14 Suppl (Suppl): S131–151.

Jasmonic Acid Dynamics, Signaling and Functions in Plants

Surya E. V.

PG Department of Botany, Korambayil Ahamed Haji Memorial
Unity Women's College, Narukara, Manjeri, Malappuram,
676122, Kerala, India.
Email: suryaev7@gmail.com

Abstract

Jasmonic acid (JA) is an endogenous growth-regulating substance, initially identified as a stress-related hormone in higher plants, they act as signal molecules, produced within plants, that occur in extremely low concentrations, control all aspects of plant growth and development, from embryogenesis, the regulation of organ size, pathogen defence, stress tolerance etc. Similarly, the exogenous application of jasmonic acid also has a regulatory effect on plants. Abiotic stress often causes large-scale plant damage. In this review, we focus on the jasmonic acid signalling pathways in response to abiotic stresses, including cold, drought, salinity, heavy metals, and light. On the other hand, jasmonic acid does not play an independent regulatory role, but works in a complex signal network with other phytohormone signaling pathways. in response to abiotic stress. In jasmonic acid signaling pathway, the JAZ-MYC module plays a central role in the JA signaling pathway through integration of regulatory transcription factors and related genes. Simultaneously, jasmonic acid has synergistic and antagonistic effects with abscisic acid (ABA), ethylene (ET), salicylic acid (SA), and other plant hormones in the process of resisting environmental stress.

Keywords: Antagonistic, Signaling, Jasmonic acid, Synergistic.

1. Introduction

Jasmonic acid is a plant hormone that plays a key role in regulating plant growth, development and response to environmental stresses. Jasmonic acid and its derivatives play a crucial role in a plant's defence against both biotic and abiotic stresses. The functions performed by jasmonic acids in protection growth and mobilizing plant defense responses constitute a direct path for stress reduction. It is a lipid derived plant hormone that belongs to the oxylipin family of compounds. The chemical structure of jasmonic acid consists of a pentanoic acid side chain with a cyclopentanone ring and a carboxylic acid group. It is involved in signalling pathways that control processes such as seed germination, root growth, flower development and defence against herbivores and pathogens. When plants are under stress, such as from insect attack or physical damage, they produce jasmonic acid as part of their defence response. This hormone triggers the production of defensive compounds, such as toxins and volatile organic compounds, that can deter herbivores and attract predators of the herbivores (Ruan et al. 2019).

2. Structure of jasmonic acid

Jasmonic acid is a lipid derived plant hormone that belongs to the oxylipin family of compounds. It is derived from the fatty acid linolenic acid and contains a cyclopentanone ring. The chemical structure of jasmonic acid consists of a pentanoic acid side chain with a cyclopentanone ring and a carboxylic acid group. Its chemical structure is 3-oxo-2-2′-cis-pentenyl-cyclopentane-1-acetic acid, which is the core of the jasmonic acid structure. Jasmonic acid is an oxo monocarboxylic acid with a (3-oxocyclopentyl) acetic acid substituted by a (2Z)-pent-2-en-1-yl group at position 2 of the cyclopentane ring (Fig. 1).

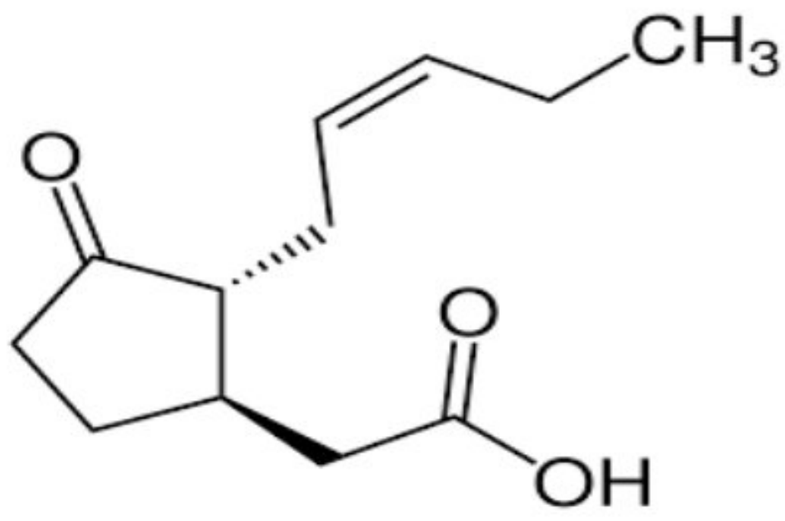

Figure 1. Structure of jasmonic acid

3. Biosynthesis

There are three pathways for the synthesis of jasmonic acids, including the octadecane pathway starting from linolenic acid and the hexadecane pathway starting from hexadecatrienoic acid (Fig. 2). All three pathways require three reaction sites: the chloroplast, peroxisome, and cytoplasm (Taiz et al. 2015).

Biosynthesis of jasmonic acids has been studied in a variety of monocotyledonous and dicotyledonous plants during the last decades. Most of the work is done in the model plants *Arabidopsis thaliana* and *Lycopersicon esculentum* (tomato) (Wasternack and Song 2017). So far, various enzymes in the jasmonic acids synthetic pathway have been identified, and our knowledge of the relationship between the jasmonic acid synthesis pathway and other metabolic pathways is gradually improving. In *Arabidopsis*, there are three pathways for the synthesis of jasmonic acids, including the octadecane pathway starting from α-linolenic acid and the hexadecane pathway starting from hexadecatrienoic acid. All three pathways require three reaction sites: the chloroplast, peroxisome, and cytoplasm. The synthesis of 12-oxo-phytodienoic acid (12-OPDA) or deoxy methylated vegetable dienic acid (dn-OPDA) from unsaturated fatty acid takes place in the chloroplast, which is then converted to jasmonic acid in the

peroxisome. In the cytoplasm, jasmonic acid is metabolized into different structures by various chemical reactions, such as MeJA (methyl jasmonate), JA-Ile (jasmonyl isoleucine), *cis*-jasmone (CJ), and 12-hydroxyjasmonic acid (Ghorbel et al. 2021).

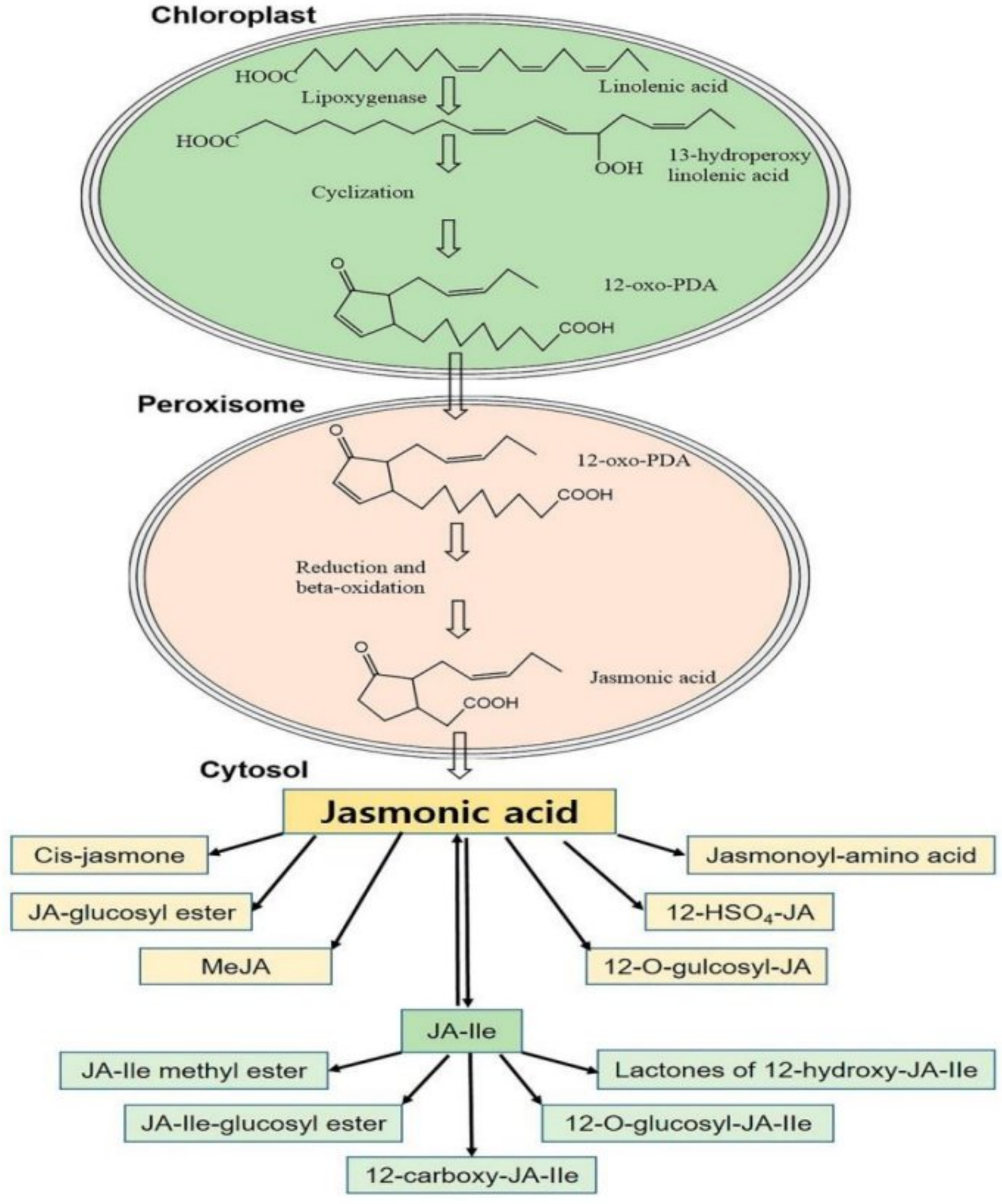

Figure 2. In chloroplast, the precursor of jasmonic acid called linolenic acid is converted into 13- hydroperoxyl linolenic acid by lipoxygenase when plant undergo any abiotic stress conditions. 13- hydroperoxyl linolenic acid undergo cyclisation and reduction it forms 12- oxo phytodienoic acid (12-OPDA). 12 OPDA enters into peroxisome where it undergoes reduction and beta oxidation form jasmonic acid. In cytosol JA undergo various metabolic processes and forms cis- jasmone, jasmonic acid glucosyl esters (JA-

glucosyl ester), Methyl jasmonate (MeJA), jasmonic acid isoleucine methyl ester (JA-Ile methyl ester), jasmonic acid isoleucine glucosyl ester (JA-Ile-glucosyl ester), 12- carboxy- jasmonic acid isoleucine (12- carboxy JA Ile), 12-o- glucosyl jasmonic acid isoleucine (12-o-JA-Ile), lactones of 12-hydroxy –JA- Ile, 12-o- glucosyl jasmonic acid, 12- H2SO4-jasmonic acid and jasmonyl amino acids (Wang et al. 2020).

4. Transmission of signal

The defence response triggered by a signal can result in a local defence response near the wound, a systemic acquired resistance (SAR) at the uninjured site, and even induced defence responses from adjacent plants. Wounding induces expression of genes encoding defense related proteins involved in wound healing in these defence responses, short-distance transmission and long-distance transmission of jasmonic acid signals are involved. With the studies in the area of mechanisms of hormone signalling networks, it has been found that salicylic acid, ethylene, auxin, and other plant hormones interact with jasmonic acid to regulate plant adaptation to the environment. At present, the understanding of complex regulatory networks and metabolic processes after plants perceive environmental signals is still very limited (Hu et al. 2009).

Jasmonic acid not only activates defence-related genes, it also shuts down growth. Jasmonic acid induces growth suppression allows relocation of resources to metabolic pathways involved defence. Jasmonic acid acts through a conserved ubiquitin ligase-based signalling mechanism that bears close resemblance to those described for auxin and gibberellin. Although unconjugated jasmonic acid is hormonally active, many jasmonic acid response require activation of the hormone for optimal activity by conversion into amino acid conjugate, such as jasmonic acid – isoleucine (JA-Ile). This

conjugation is performed by enzyme referred to as jasmonic acid resistance (JAR) proteins, which belongs to a family of carboxylic acid conjugating enzymes. JAR1 for example, exhibits a high substrate specificity for jasmonic acid and isoleucine and appears to be of particular importance of jasmonic acid defence signaling.

When levels of bioactive jasmonic acid are low, the expression of jasmonate-responsive genes is repressed by members of the JAZ (Jasmonate ZIM domain) protein family which are key regulators of the jasmonic acid response. JAZ repressors act by binding to the MYC2 transcription factor, a major switch in the activation of jasmonic acid dependent genes. JAZ repressors also maintain the chromatin in a closed state that prevents jasmonic acid responsive transcription factors from binding to their targets to maintain chromatin in the inactive state, JAZ proteins bind to the F box protein COI1, which is an essential component of the SCF protein complex SCFCOI1, a multiprotein E3 ubiquitin ligase, two additional proteins and two histone deacetylase enzymes (HDAC6 and HDA19) act as co repressors along with the JAZ-COI1 complex and are instrumental in maintaining the chromatin in an inactive state. The binding of JA-Ile to the JAZ-COi1 co receptors leads to the ubiquitination of JAZ by the SCF COI1-JA-Ile complex, followed by JAZ degradation of JAZ liberates the MYC transcription factor, which then recruits various other chromatin remodelling proteins and transcription factors that bring about the expression of the early jasmonic acid responsive genes (Li et al. 2021).

4.1 Short distance signal transmission

In plants, mechanical damage or insect feeding can cause rapid and transient accumulation of jasmonic acid and JA-Ile at the site of injury,

thereby activating the expression of defence genes surrounding the wound and producing a local defence response. Three well known phytohormones, salicylic acid, jasmonic acid and ethylene are central in regulation of different signaling pathways in plant defense to distinct pathogens. In the local defence response, there are two ways of short-distance transmission of the jasmonic acid signal. First, the systemin produced by the wounding acts as a signalling molecule, which is transmitted to the adjacent site through the apoplast and phloem to activate the jasmonic acid cascade reaction pathway. Second, jasmonic acid and JA-Ile induced by systemin act as signals and are transported to adjacent sites for defensive responses (Turner et al. 2002; Kazan and Manners 2008).

4.2 Long distance signal transmission

Long-distance transmission of jasmonic acid signals is mainly via airborne transmission. Some works has also shown that jasmonic acids are not simply transported along the vascular bundle, but are accompanied by resynthesis of jasmonic acids during transport even jasmonic acid signaling and defence gene expression are systemically activated within hours. The localization of various jasmonic acid synthetases (such as LOX, AOS, etc.) was also found in the companion cell–sieve element complex (CC-SE) of tomato vascular bundles, and the sieve molecules in the phloem have the ability to form the jasmonic acid precursor OPDA (phytodienoic acid). Communication between salicylic acid and JA dependent defence signaling pathways has been identified. Other advances in induced resistance signaling, such as the implication that ethylene is involved in the generation of systemic signal molecules, the suggestion of the involvement of lipid derived molecules in long distance signaling and the identification of new components of various systemic defense signaling pathways, shed new lights

on how plants actively defend themselves against harmful organisms (Turner et al. 2002).

A large number of studies showed that in addition to vascular bundle transmission, there are other long-distance transmission routes for jasmonic acid signals. Compared with jasmonic acid, which has difficulty in penetrating the cell membrane without carrier assistance, jasmonic acid easily penetrates the cell membrane and has strong volatility, and thus can be spread by airborne diffusion to distant leaves and adjacent plants. It has been confirmed in a range of plants, such as *Arabidopsis thaliana*, *Nicotiana tabacum*, *Phaseolus lunatus*, and *Artemisia kawakamii*, that JA can be transmitted by air between damaged and undamaged leaves or between adjacent plants (Thaler et al. 2002).

4.3 Jasmonic acid receptor

In a screen for delayed floral organ abscission in *Arabidopsis*, a novel mutant of CORONATINE INSENSITIVE1 (COI1), the F box protein that has been shown to be jasmonic acid co receptor. The ABC transporter AtJAT1/AtABCG16 with jasmonic acids transport ability was screened by a yeast system. Radioactive isotope uptake experiments and autoradiography experiments showed that AtJAT1/AtABCG16 acts as a high-affinity transporter to regulate the subcellular distribution of JAs. AtJAT1/AtABCG16 is localized on the nuclear and plasma membranes of plant cells and mediates the transport of jasmonic acids across the plasma membrane and the bioactive JA-Ile across the inner membrane of the nuclear membrane to activate JA responses at low concentration. When the concentration of jasmonic acids is high, the function of the jasmonic acid transporter on the cytoplasmic membrane is dominant, which reduces the intracellular jasmonic acid and JA-Ile concentrations to desensitize the

jasmonic acid signal. The jasmonic acids signalling pathway is activated in other cells by transporting jasmonic acid to the apoplast. AtJAT1/AtABCG16 can regulate the dynamics of JA/JA-Ile in cells, which leads to the quick transport of JA-Ile into the nucleus when the plant is under stress, as well as the quick desensitization of the JA signal to avoid the inhibition of plant growth and development by the defence response (Kim et al. 2013).

The understanding of jasmonic acid receptors has undergone a complex process. In 1994, Feys first found that the *Arabidopsis coronatine insensitive1* (*coi1*) mutant lost all responses to jasmonic acid, and further studies indicated that the *COI1* gene encodes an F-box protein that is a component of E3 ubiquitin ligase. In this case, COI1 associates with the SKP1 protein and Cullin protein to form the SCF-type E3 ubiquitin ligase that is referred to as SCF^{COI1}, which targets the repressor proteins for degradation by ubiquitination. The outburst of COI1 protein is of great significance for the study of the jasmonic acid signaling pathway (Singh and Jwa 2013).

It was once thought that COI1 is the receptor for jasmonic acid signalling in cells, until the discovery of a jasmonate Zinc finger Inflorescence Meristem (ZIM)-domain (JAZ) protein family, which gave a new understanding of the jasmonic acid signal transduction pathway. In 2007, three research groups simultaneously found that JAZ proteins act as repressors in the jasmonic acid signalling pathway. To date, 13 JAZ proteins have been found in *Arabidopsis*, most of which have two conserved domains, Jas and ZIM. The JAZ protein interacts with COI1 via the Jas domain and interacts with MYC2 via the ZIM domain. Therefore, many researchers believe that JAZ proteins are the target protein of COI1 and the degradation of JAZ proteins is a key step to relieve the inhibition of the

jasmonic acids pathway. However, in 2011, Sheard et al. proposed different views on jasmonic acids receptors through the analysis of crystal structure and confirmed that the COI1–JAZ complex is a high-affinity receptor for the bioactive JA-Ile; that is, COI1 and JAZ are coreceptors of jasmonic acid signaling. It is currently believed that plants perceive stimuli from the external environment to generate JA-Ile, which promotes the interaction between COI1 and JAZ proteins. Subsequently, JAZ proteins are degraded after being transferred to the 26S proteasome, and simultaneously, transcription factors (TFs) are released to activate the expression of downstream genes (Sheard et al. 2011).

4.3.1 Jasmonic acid transcription factor

JA-Ile activates the MYC transcription factors by directly binding to JAZ and COI1, which results in the degradation of JAZ through the 26S proteasome pathway. Recent studies have shown that the MYB transcription factors also bind with JAZ repressors and can be activated by the degradation of JAZ in the presence of JA-Ile. In addition, several other transcription factors (TFs) such as NAC, ERF, and WRKY are also involved in the jasmonic acids signaling. These JA-responsive TFs regulate the expression of many genes involved in the growth and development of plants, and especially the responses and adaptation of plants to the environment. Studies have also shown that jasmonic acid signaling can also induce the MAP kinase cascade pathway, calcium channel, and many processes that interact with signalling molecules such as ethylene, salicylic acid, and abscisic acid to regulate plant growth and development (Heitz et al. 2016; Ali and Baek 2020).

4.3.2 MYC transcription factor

The basic helix–loop–helix (bHLH) transcription factor MYC2 is a well-known regulatory protein encoded by the *JIN1* gene. Most members of the JAZ protein family interact with MYC2. For a long time, it was believed that only the MYC2 protein can directly interact with the JAZ protein. Two other bHLH proteins, MYC3 and MYC4, share high sequence similarity with MYC2, suggesting they probably have similar functions. Indeed, MYC3 and MYC4 interact with JAZ proteins in vivo and in vitro, have similar DNA-binding specificity to MYC2, and act synergistically and distinctly with MYC2. A closely related TF, MYC5 (bHLH28), is induced by jasmonic acids and required for male fertility. Besides transcriptional activators, JA-associated MYC2-like (JAM) proteins, JAM1, JAM2, and JAM3, were discovered as transcriptional repressors via forming protein–protein interactions with JAZs to regulate jasmonic acids responses (Sasaki-Sekimoto et al. 2014).

4.3.3 MYB Transcription factor

Most of the jasmonic acids -responsive MYB TFs belong to the R2R3-MYB family, which are widely distributed in plants and required for many processes. MYB51 and MYB34 regulate the synthesis of tryptophan and glucosinolates and act downstream of MYC2. However, many studies have found that MYB TFs can directly bind to JAZ proteins, indicating the release from JAZs to activate their target genes. For instance, in *Arabidopsis*, MYB21 and MYB24 are key factors in stamen and pollen maturation and MYB75 can positively regulate the anthocyanin accumulation and trichome initiation. Recently, a set of MYB TFs, MYB11, MYB13, MYB14, MYB15, and MYB16, were identified as repressors in the regulation of rutin biosynthesis in buckwheat (Ruan et al. 2019).

4.3.4 NAC transcription factor

ATAF1 and ATAF2 TFs in the *Arabidopsis* NAC family are both induced by jasmonic acid signaling and involved in plant resistance to drought, salt stress, *Botrytis cinerea*, and other pathogens. At the same time, ATAF1 and ATAF2 have an important regulatory effect on oxidative stress, flowering, and pod development of plants. Two other NAC TFs in *Arabidopsis*, ANAC019 and ANAC055, are also present downstream of the MYC2 protein and regulate seed germination, cell division, and the synthesis of secondary walls of cells. In addition, ATAF1, ATAF2, ANAC019, and ANAC055 are also involved in the crosstalk between jasmonic acid and salicylic acid signaling pathways (Fraga et al. 2021).

4.3.5 Ethylene responsive transcription factor

Microarray experiments at the genetic level have confirmed that jasmonic acid signaling can induce the transcription of many *ERF* genes. The first evidence for a link between AP2/ERF TFs and jasmonic acid signaling was found in *Catharanthus roseus*. The jasmonic acids-induced ORCA proteins, ORCA2 and ORCA3, belong to the AP2/ERF-domain family and can activate the expression of monoterpenoid indole alkaloid biosynthesis genes. Based on the observation of ORCAs, the *Arabidopsis* ERF proteins, ERF1 and ORA59, function dependently on jasmonic acids and/or ET for the defenses against *Botrytis cinerea*. Moreover, ORA59, rather than ERF1, acts as the integrator of JAs and ET signals and regulates the biosynthesis of hydroxycinnamic acid amides. The JAs-induced ORA47 can activate the expression of the jasmonic acids biosynthesis gene *AOC2*, indicating that ORA47 might act as an important regulator in the positive jasmonic acids-responsive feedback loop. Moreover, jasmonic acids-responsive AtERF3 and AtERF4 act as repressors by not only down regulating their target genes'

expression, but also interfering with the activity of other activators. Interestingly, the activity of above TFs is not directly repressed by JAZ proteins, suggesting the presence of adaptors or corepressors in the jasmonic acid signaling pathway (Gan et al. 2007).

4.3.6 WRKY transcription factor

WRKY transcription factors play an important regulatory role in plant development, senescence, and coping with environmental stress. In *Arabidopsis*, there are 89 members in the WRKY transcription factor family. It has been shown that some WRKY TFs are regulated by the jasmonic acid signaling pathway, such as WRKY70, WRKY22, WRKY50, WRKY57, and WRKY89. These WRKY transcription factors are mostly associated with plant defense functions. In *Nicotiana attenuata*, two WRKY transcription factors, NaWRKY3 and NaWRKY6, regulate the expression of jasmonic acids biosynthesis-related genes (*LOX*, *AOS*, *AOC*, and *OPR*) to increase the levels of jasmonic acid and JA-Ile. In addition, *Arabidopsis* WRKY57 interacts with the inhibitor JAZ4/JAZ8 in the jasmonic acid signaling pathway and the inhibitor IAA29 in the auxin signaling pathway, thereby regulating the interaction between jasmonic acid and auxin-mediated signaling pathways and effects on plant leaf senescence (Jiang et al. 2014).

5. Functions of jasmonic acid

5.1 Regulating plant responses to abiotic and biotic stresses as well as plant growth and development

Jasmonic acid is a plant-signalling molecule closely associated with plant resistance to abiotic stress. In abiotic stress, JA is usually involved in physiological and molecular responses. Physiological responses often

include accumulation of amino acids (isoleucine and methionine) and soluble sugars, activation of the antioxidant system (superoxide anion radical, peroxidase, NADPH-oxidase) and regulation of stomatal opening and closing. Molecular responses often involve the expression of jasmonic acid-associated genes (*JAZ, AOS1, AOC, LOX2,* and *COI1*), interactions with other plant hormones (ABA, ET, SA, GA, IAA, and BR), and interactions with transcription factors (MYC2 and bHLH148) (Taiz et al. 2015).

5.2 Growth inhibition

Jasmonic acid has been shown to inhibit primary growth by reducing cell division in the meristem zone and inhibiting cell elongation in the elongation zone.

5.3 Senescence

Exogenous application of jasmonic acid caused premature senescence in attached and detached leaves such as *Arabidopsis*, the jasmonic acid-signaling pathway is required for jasmonic acid to promote leaf senescence. Jasmonic acid levels in senescing leaves are 4-fold higher than in non-senescing ones. Concurrent with the increase in jasmonic acid level in senescing leaves, genes encoding the enzymes that catalyse most of the reactions of the jasmonic acid biosynthetic pathway are differentially activated during leaf senescence. Both jasmonic acid and H_2O_2 are two crucial signalling molecules positively regulating leaf senescence, whereas whether and how they are coordinated in leaf senescence remains elusive. Here, we report that H_2O_2 accumulates in jasmonic acid-treated leaves, while scavenging the increased H_2O_2 can significantly suppresses jasmonic acid-induced leaf senescence and the expression of *senescence-associated genes* (*SAGs*). The mutant *myc2* with a mutation of *MYC2*, a master

transcription factor in JA signalling pathway, exhibits delayed leaf senescence with increased catalase activity and decreased H_2O_2 accumulation compared with the wild type upon jasmonic acid treatment. Further study showed that MYC2 downregulates *CATALASE 2* (*CAT2*) expression by binding to its promoter, thus promoting jasmonic acid-induced H_2O_2 accumulation and leaf senescence. Moreover, the delayed leaf senescence with reduced H_2O_2 accumulation and *SAGs* expression of the *myc2* mutant is significantly reverted by the *cat2-1* mutation in *myc2 cat2-1* double mutant. Thus, promoting leaf senescence in a MYC2 dependent manner in Arabidopsis (Zhang et al. 2020).

5.4 Tendril coiling

A coiling-inducing factor was isolated from some tendrils and identified by nuclear magnetic resonance and mass spectrometry. When applied to detached tendrils, exogenous α-linolenic acid, but not linoleic acid or oleic acid, induced tendril coiling. Further investigations showed that metabolites of α-linolenic acid, jasmonic acid and, even more so, methyl jasmonate, are highly effective inducers of tendril coiling. Methyl jasmonate was most active when administered by air and, in atmospheric concentrations as low as 40–80 nM, induced a full free-coiling response with similar to mechanical stimulation. Methyl jasmonate could be one of the endogenous chemical signals produced in mechanically stimulated parts of a tendril and, being highly volatile, act as a diffusible gaseous mediator spreading through the intracellular spaces to trigger free coiling of tendrils (Kim et al. 2013).

5.5 Flower development and leaf abscission

Development of inflorescences and flowers in plants is controlled by the combined action of environmental and genetic signals. Investigations reveal that the phytohormone jasmonate plays a critical function in plant reproduction such as male fertility, sex determination and seed maturation. Jasmonic acid promoted the abscission of bean petiole explants via the degradation of cell wall polysaccharides in the abscission zone (Taiz et al. 2015).

5.6 Initiates the production of defence proteins that inhibit herbivore digestion

Jasmonic acid initiates the production of defence proteins, most of the proteins interfere with the herbivore digestive system, some legumes synthesise alpha amylase inhibitors, which block the action of the starch digesting enzyme a-amylase. Some others produce lectins which bind to the epithelial cell line of the digestive tract and interfere with the nutrient absorption by the herbivore. A more direct attack on the insect herbivore's digestive system is performed by some plants through the production of a specific cysteine protease, which disrupt the peritrophic membrane that protects the gut epithelium of many insects. While none of these genes are essential for the vegetative growth of the plant, they have likely evolved from normal "housekeeping" genes during the coevolution of plants and their insect herbivores. The best-known antidigestive proteins in plants are the proteinase inhibitors. Found in legumes, tomato, and other plants, these substances block the action of herbivore proteolytic enzymes. After entering the herbivore's digestive tract, they hinder protein digestion by binding

tightly and specifically to the active site of protein hydrolysing enzymes such as trypsin and chymotrypsin. Insects that feed on plants containing proteinase inhibitors suffer reduced rates of growth and development that can be offset by supplemental amino acids in their diet. The defensive role of proteinase inhibitors has been confirmed by experiments with transgenic tobacco. Plants that had been transformed to accumulate increased levels of proteinase inhibitors suffered less damage from insect herbivores than did untransformed control plants (Heitz et al. 2016).

6. Conclusion

Jasmonic acid is a lipid derived plant hormone that orchestrates a wide range of physiological processes through its intricate signaling pathways. Its signaling pathways is complex and involves interaction with other hormones and signaling molecules. It is a crucial regulator of plant growth, development and responses to environmental cues, including biotic and abiotic stresses. Jasmonic acid functions in plant defence mechanism against herbivores, pathogens and adverse environmental conditions, highlighting its significance in plant survival and adaptation. The complex interplay of jasmonic acid with other hormone and signaing molecules underscore its versatile role in shaping plant responses to diverse stimuli. Further research on jasmonic acid signaling and its functions in plants could provide valuable insights into improving crop productivity and resilience in the face of changing environmental conditions, it also holds promise for enhancing our understanding of plant biology. The studies on jasmonic acid made great progress, and the jasmonic acid signal transduction pathway has also been established, but there are still many questions regarding the regulatory process which need to be answered.

References

Ali MS, Baek KH (2020) Jasmonic acid signaling pathway in response to abiotic stresses in plants. Int J Mol Sci 21(2): 621.

Fraga OT, de Melo BP, Quadros IPS, Reis PAB, Fontes EPB (2021) Senescence-associated glycine max (Gm)NAC genes: Integration of natural and stress-induced leaf senescence. Int J Mol Sci 22(15): 8287.

Gan Y, Yu H, Peng J, Broun P (2007) Genetic and molecular regulation by DELLA proteins of trichome development in *Arabidopsis*. Plant Physiol 145(3): 1031–1042.

Ghorbel, M, Brini, F, Sharma, A (2021) Role of jasmonic acid in plants: the molecular point of view. Plant Cell Rep 40: 1471–1494.

Heitz T, Smirnova E, Widemann E, Aubert Y, Pinot F, Ménard R (2016) The rise and fall of jasmonate biological activities. Springer, Cham, 86: 326.

Hu X., Wansha L, Chen Q, Yang Y (2009) Early signals transduction linking the synthesis of jasmonic acid in plant. Plant Signal Behav 4(8): 696–697.

Thaler JS, Farag MA, Pare PW, Dicke M (2002) Jasmonate deficient plants have reduced direct and indirect defences against herbivores. Ecol Lett 5(6): 764–774.

Jiang Y, Liang G, Yang S, Yu D (2014) *Arabidopsis* WRKY57 functions as a node of convergence for jasmonic acid-and auxin-mediated signaling in jasmonic acid–induced leaf senescence. The Plant Cell Rep 26 (1): 230–245.

Kazan K, Manners JM (2008) Jasmonate signaling: toward an integrated view. Plant Physiol 146(4): 1459–1468.

Kim J, Dotson B, Rey C, Lindsey J, Bleecker AB, Binder BM (2013) New clothes for the jasmonic acid receptor COI1: Delayed abscission, meristem arrest and apical dominance. PLoS One 8(4): 505.

Li M, Yu G, Cao C, Liu P (2021) Metabolism, signaling, and transport of jasmonates. Plant Commun 2(5): 100231.

Ruan J, Zhou Y, Zhou M, Yan J, Khurshid M, Weng W, Cheng J, Zhang K (2019) Jasmonic acid signaling pathway in plants. Int J Mol Sci 20: 2479.

Sasaki-Sekimoto Y, Saito H, Masuda S, Shirasu K, Ohta H (2014) Comprehensive analysis of protein interactions between JAZ proteins and bHLH transcription factors that negatively regulate jasmonate signaling. Plant Signal Behav 9(1): 276.

Sheard LB, Tan X, Mao H (2011) jasmonate perception by inositol phosphate-potentiated COI1-JAZ co receptor. Plant cell Rep 24(2): 536–550.

Singh R, Jwa NS (2013) The rice MAPKK–MAPK interactome: the biological significance of MAPK components in hormone signal transduction. Plant Cell Rep 32: 923–931.

Taiz L, Zeiger E, Møller IM, Murphy A (2015) Plant physiology and development. 6th ed. Sinauer Associates, Sunderland, CT, USA, pp 433–436.

Turner JG, Ellis C, Devoto A (2002) The jasmonate signal pathway. Plant Cell 14(suppl 1), S153–S164.

Wang J, Song L, Gong X, Xu J, Li M (2020) Functions of jasmonic acid in plant regulation and response to abiotic stress. Int J Mol Sci 21: 1446.

Wasternack C, Song S (2017) Jasmonates: biosynthesis, metabolism, and signaling by proteins activating and repressing transcription. J Exp Bot 68(6): 1303–1321.

Chapter 7: Jasmonic Acid Dynamics, Signaling and Functions in Plants

Zhang Y, Ji TT, Li TT, Tian YY, Wang LF, Liu WC (2020) Jasmonic acid promotes leaf senescence through MYC2-mediated repression of CATALASE2 expression in *Arabidopsis*. Plant Sci 299: 367.

Brassinosteroids in Plants

Sudheesha K.

PG Department of Botany, Korambayil Ahamed Haji Memorial
Unity Women's College, Narukara, Manjeri, Malappuram,
676122, Kerala, India.
Email: sudheeshasudhi212@gmail.com

Abstract

Brassinosteroids (BRs) are a unique class of plant hormones, that are essential for normal plant growth. Brassinosteroids are endogenous plant hormones essential for the proper regulation of multiple physiological processes required for normal plant growth and development. Since their discovery more than 30 years ago, extensive research on the mechanisms of BR action using biochemistry, mutant studies, proteomics and genome-wide transcriptome analyses, has helped refine the brassinosteroid biosynthetic pathway, identify the basic molecular components required to relay the brassinosteroid signal from perception to gene regulation, and expand the known physiological responses influenced by brassinosteroids. These mechanistic advances have helped answer the intriguing question of how brassinosteroids can have such dramatic pleiotropic effects on a broad range of diverse developmental pathways and have further pointed to brassinosteroid interactions with other plant hormones and environmental cues. Moreover, brassinosteroids physiological responses including cell elongation and division, vascular differentiation, flowering, pollen development and photomorphogenesis.

Keywords: Brassinosteroids, Photosystem, Phytohormones, Signaling.

Chapter 8: Brassinosteroids in Plants

1. Introduction

Brassinosteroids (BRs), inherent plant hormones with widespread presence, play a pivotal role in promoting growth by impacting cell expansion and proliferation. Brassinosteroids are known to be naturally occurring polyhydroxylated plant steroids that showed diverse roles in regulation of various physiological and developmental processes in plants under both natural and stressful conditions. In the recent past brassinosteroids have shown the ability to cross talk with other phytohormones such as auxin, polyamines, cytokinin, ethylene, and jasmonic acid in regulating varied ranges of physiological and biochemical processes in plants. On the other hand, the exogenous application of brassinosteroids in agriculture to improve growth and yield under various stress conditions including drought, salinity, extreme temperatures, and heavy metal toxicity is of immense significance as these stresses severely hamper the normal metabolism of plants.

The information available till date regarding brassinosteroids will definitely help in establishing various mechanisms which modulate various processes in plants and overcome the future challenges in agriculture. Brassinosteroid also plays pivotal role in promotion of cell expansion, cell elongation, cell division, and vascular differentiation, and provides protection against various abiotic and biotic stresses. Mutant plants lacking proper brassinosteroid biosynthesis and signaling display distinct phenotypes, underscoring the crucial role of these hormones as regulators in fundamental physiological processes. This includes organ elongation, vascular differentiation, male fertility, senescence timing, and leaf development (Fridman and Savaldi-Goldstein 2013).

2. Structure of brassinosteroids

Brassinosteroids is a polyhydroxylated derivative of 5α-cholestan, namely (22*R*, 23*R*, 24S)-2α,3α,22,23-tetrahydroxy-24-methyl-B-homo-7-oxa-5α-cholestan-6-one (Fig. 1). Thus, plants possess a growth-promoting steroid with structural similarity to cholesterol-derived animal steroid hormones such as androgens, estrogens and corticosteroids from vertebrates, and ecdysteroids from insects and Crustacea. The brassinosteroid family consists of BL and about 68 other free brassinosteroids plus several conjugates (Fujioka and Yokota 2003). These differ from BL by variations at C-2 and C-3 in the A ring; the presence of a lactone, ketone, or de-oxo function at C-6 in the B ring; the stereochemistry of the hydroxyl groups in the side chain, and the presence or absence of a methyl (methylene) or ethyl (ethylene) group at C-24. The conjugates are glycosylated, meristylated and laurylated derivatives of the hydroxyls in ring A or in the side chain. Many of the known brassinosteroids are biosynthetic precursors or metabolic products of BL, although castasterone, the immediate precursor of BL, is believed to have independent biological activity in some plants. The optimal structure for highest brassinosteroid activity normally is that found in BL, consisting of a lactone function at C-6/C-7, cis-vicinal hydroxyls at C-2 and C-3, *R* configuration of the hydroxyls at C-22/C-23 and a methyl substitution at C-24 (Mandava 1988).

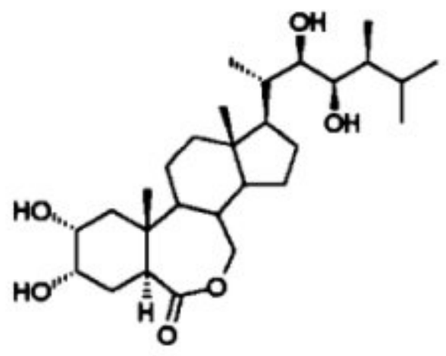

Figure 1. Chemical structure of brassinosteroids.

3. Biosynthesis of brassinosteroids

Brassinosteroids, like animal and insect steroids originating from cholesterol, undergo transformation from plant sterols. Teasterone, typhasterol, and castasterone, among natural BRs, are considered precursors of brassinolide. These analogs, including brassinolide, are metabolized from campesterol, a common plant sterol with a similar carbon skeleton in the side chain (Akira et al. 1997). Brassinosteroids are naturally occurring polyhydroxylated steroidal phytohormones crucial for normal plant growth and development. They fall into three categories (C27, C28, or C29 steroids) based on their C-24 alkyl substituents. Among the identified BRs, brassinolide (BL) stands out as the most biologically active compound, widely present in numerous plant species. BL, a 28-carbon compound with an S-methyl group at C24, is a key focus in brassinosteroid research. Other brassinosteroids typically represent intermediates or inactivated products resulting from various catabolic reactions.

The biosynthesis of BL Involves two parallel pathways: the early and late C-6 oxidation pathways. These pathways start with the brassinosteroid-specific biosynthetic precursor campesterol (CR). In the early C-6 oxidation pathway, CN (campestanol) undergoes C-6 oxidation before DWF4-mediated C-22 hydroxylation, leading to intermediates like 6-oxocampestanol (6-oxoCN), cathasterone (CT), teasterone (TE), 3-dehydroteasterone (3DT), typhasterol (TY), and ultimately castasterone (CS). In contrast, the late C-6 oxidation pathway involves C-22 hydroxylation prior to C-6 oxidation. DWF4 hydroxylates CR at C-22, forming intermediates like 6-deoxocathasterone (6-deoxoCT), which then proceed through the late C-6 oxidation pathway. In crop plants such as tomato and tobacco, the late C-6 oxidation pathway predominates, resulting

in endogenous brassinosteroids primarily from this pathway. Notably, in *Arabidopsis*, BR6ox connects the late and early C-6 oxidation pathways, and DWF4 can act independently of campestanol. The pathways can branch at campesterol, establishing an early C-22 hydroxylation pathway (Fujioka and Yokota 2003).

4. Signal transduction of brassinosteroids

RLKs (Receptor-like serine/ threonine kinases, the predominant group of plant receptor kinases, are membrane-bound proteins with extracellular ligand-binding domains and cytoplasmic kinase domains. They transmit signals by phosphorylating serine, threonine, or, in some cases, tyrosine residues of target proteins. RLKs respond to various ligands, including signals from biotic sources and plant hormones like brassinosteroids, auxin, and peptides. In the brassinosteroids signaling pathway, RLKs employ signal amplification and repressor inactivation strategies, translating extracellular hormone signals into transcriptional responses within the cell. When brassinolide binds to the receptor kinase BRI1(BRASSINOSTEROID-INSENSITIVE1), on the plasma membrane, it initiates a phosphorylation cascade. This cascade leads to the inactivation of the repressor protein BIN2 (BRASSINOSTEROID-INSENSITIVE2). As a result, transcription factors BES1 (BRI1-EMS SUPPRESSOR1) and BZR1 (BRASSINAZOLE-RESISTANT1) become activated, leading to the expression of specific genes and the propagation of the BR-mediated signal for cellular responses. The BRI1 receptor, part of the plasma membrane LRR subfamily of RLKs, consists of an extracellular domain binding brassinolide, a transmembrane domain, and a cytoplasmic kinase domain with tyrosine, serine, or threonine specificity. When brassinolide binds, BRI1 homodimers

activate and form heterooligomers with BAK1. Both RLKs undergo auto- and transphosphorylation upon activation. Before brassinolide binding, BRI1 interacts with BKI1 (BRI1-KINASE INHIBITOR1), preventing association with BAK1. This interaction acts as a regulatory mechanism in the absence of the BRs hormone. Up on BRI1 activation, BKI1 is released from the plasma membrane, BRI1 and BAK1 dimerize, and BRI1 phosphorylates and activates two plasma membrane–anchored receptor like cytoplasmic kinases (RLCKs), the BR-SIGNALING KINASE1 (BSK1) and CONSTITUTIVE DIFFERENTIAL GROWTH1 (CDG1). Activated BSK1 and CDG1 then phosphorylate and activate the Serine/threonine phosphatase BRI1 SUPPRESSOR1 (BSU1). This, in turn, inactivates the repressor protein BIN2. BIN2 is a serine/threonine protein kinase that, in Absence of brassinolide, negatively regulates the closely Related transcription factors BES1 and BZR1 by phosphorylation. Phosphorylation of BES1/BZR1 by active BIN2 has at least two regulatory roles. First, BIN2-mediated phosphorylation of the transcription factors prevents them from shuttling to the nucleus and causes their retention in the cytosol. Second, phosphorylation prevents BES1/BZR1 from binding to target promoters, thus blocking Their activity as transcriptional regulators. In the presence of brassinolide, the activated phosphatase BSU1 dephosphorylates BIN2 and promotes its degradation by the 26S proteasome system, thus blocking its Activity.BES1 and BZR1 Are then dephosphorylated by PROTEIN PHOSPHATASE2A (PP2A), and the active forms of BES1 and BZR1 Move into the nucleus where they regulate the expression of brassinolide response genes (Fig. 2) (Taiz et al. 2015).

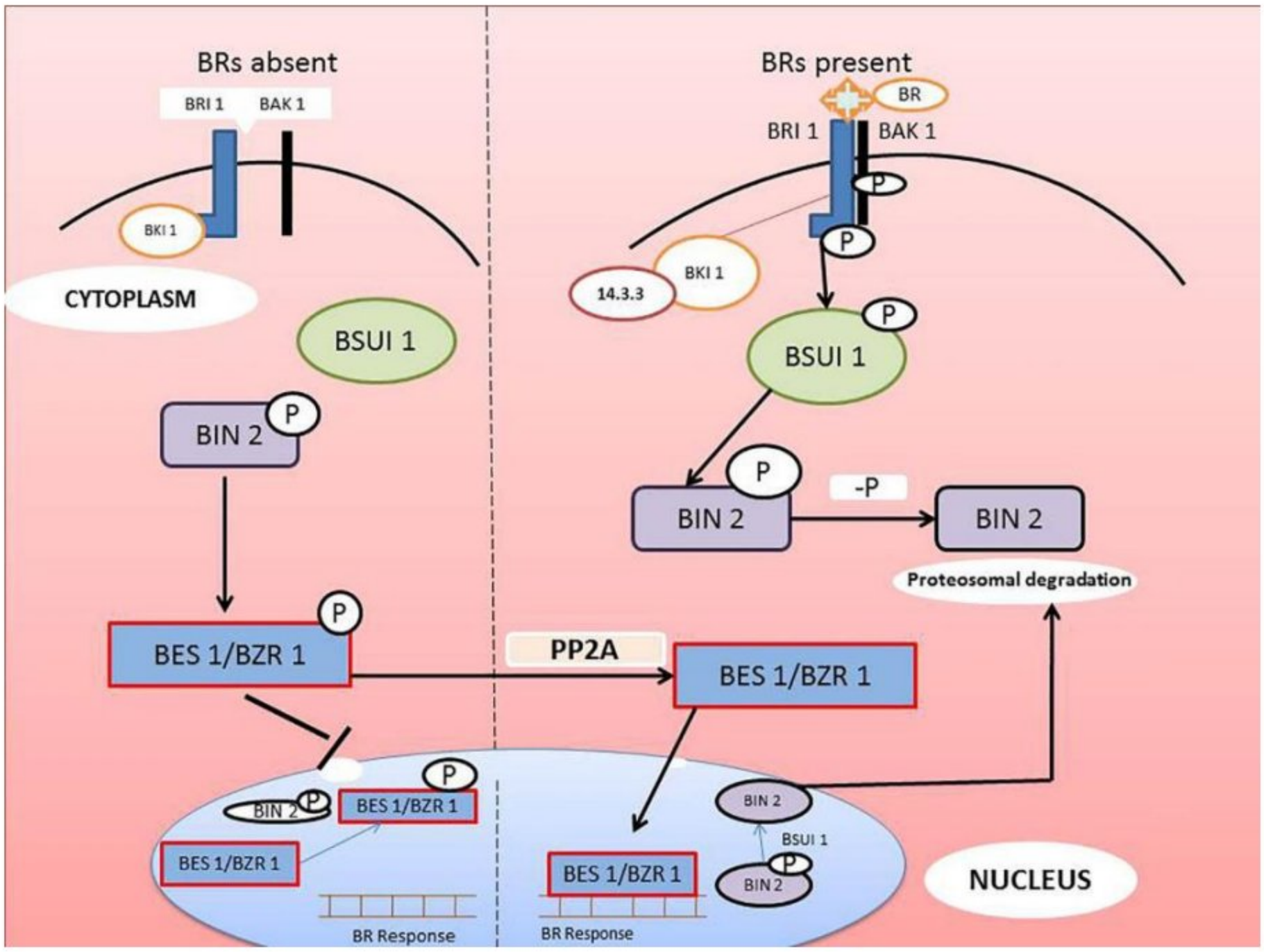

Figure 2. BR signal transduction pathway in plants. The receptor BRI1 is localized on the plasma membrane (PM). The extracellular region consists of a coiled stretch of leucine rich repeat (LRR) sequences containing an Island domain (ID) that functions as part of the Brassinolide (BL) binding site. The intracellular Portion contains a kinase domain (KD) and the C-terminal tail (CT). Signal perception occurs at the cell surface and results in changes in BR-regulated gene expression. BRRE, BRs Response element (Chung and Choe 2013).

5. Functions of brassinosteroids

Brassinosteroids exhibit diverse effects, influencing various cellular responses such as stem elongation, pollen tube growth, leaf bending and epinasty, root inhibition, induction of ethylene biosynthesis, proton-pump activation, xylem differentiation, and the regulation of gene expression.

5.1 Cell expansion and cell division

Brassinosteroids are naturally occurring compounds that stimulate growth and are present in small quantities in pollen, seeds, and young plant tissues across various plant species. Brassinosteroids play a crucial role in plant growth by influencing both cell division and cell expansion. They induce elongation in various plant structures such as hypocotyls, epicotyls, peduncles, coleoptiles, and mesocotyls. This cell expansion, triggered by brassinosteroids, involves proton extrusion and hyperpolarization of the cell membrane, thereby stimulating and accelerating the cell cycle. The plasticity of the cell wall increases as proton extrusion acidifies the apoplast, activating cell wall loosening enzymes. Brassinosteroids contribute to cell enlargement through their impact on gene expression and enzyme activity. Molecular genetic studies on mutants have provided insights into the necessity of brassinosteroids in cell elongation. In root meristem cells, a cellular analysis combined with the assessment of molecular cell cycle markers and stem cell genes reveals that brassinosteroids positively regulate the size of the root meristem. Brassinosteroids achieve this by increasing cell division, notably by elevating transcript levels of genes encoding cyclin-D3, a regulatory protein in the cell cycle. Interestingly, brassinosteroids can efficiently substitute for cytokinin in Arabidopsis callus and suspension cultures, where they promote growth (Clouse et al. 1998).

5.2 Photosynthesis

Photosynthesis is vital for both plants and life on Earth. Phytohormones are essential in guiding various stages of plant development, from organ formation to aging. They act as key regulators in growth, development, and play a central role in controlling photosynthesis.

Additionally, phytohormones are crucial for safeguarding the photosynthetic machinery during stressful conditions, providing photoprotection (Müller et al. 2021). Brassinosteroids are recognized for their ability to increase the chlorophyll content (Alyemeni et al. 2016). The role of brassinosteroids in enhancing photosynthetic rates and related attributes is widely acknowledged in various plant species. The mechanism is believed to involve the improvement of the photosynthetic carbon reduction cycle efficiency by increasing the content of related enzymes. Exogenous application of 28-homobrassinolide to wheat and mustard, or epibrassinolide to mung bean along with GA3, has demonstrated increased photosynthetic rates. Foliar spray of brassinosteroid solutions to wheat, mustard, and cucumber, as well as the application of epibrassinolide to cucumber seedlings and brassinolide to rice, have shown positive effects on CO_2 assimilation. Furthermore, 24-epibrassinolide application enhanced the light-saturated net CO_2 assimilation rate and carboxylation rate of rubisco in the Calvin cycle. Interestingly, while the epicotyl of cucumber did not respond to epibrassinolide, labeled glucose transport towards the epicotyl was favored. Additionally, foliar application of 28-homobrassinolide positively influenced Hill activity in *Vignaradiata* foliage. Epibrassinolide foliar spray increased rubisco carboxylation rate, RuBP regeneration, and quantum yield of PSII, and it's noted to recover the loss of photosynthetic apparatus from cold stress. Following HBL treatment, initial Hill reaction activity declined, but at subsequent developmental stages, there was an observed enhancement. The efficiency of Photosystem II (ϕPSII), representing the fraction of absorbed photons involved in photochemistry, serves as an indicator of overall photosynthesis. Notably,

certain studies have indicated an Increase in ϕPSII in the presence of BRs (Yu et al. 2004).

5.3 Ion homeostasis

Ion homeostasis is crucial for cell performance, maintaining optimal conditions for enzyme activity and cell signaling. Despite fluctuating external concentrations, living organisms exhibit homeostasis to regulate mineral ion levels. Salt stress disrupts ion homeostasis, impacting plant salt tolerance. Brassinosteroids play a role by positively affecting high-affinity K^+ transporters, reducing Na^+ levels, and increasing K^+ concentration, improving the K^+/Na^+ ratio. Brassinosteroids also enhance Ca^{2+}/Na^+ and K^+/Na^+ ratios in wheat cultivars, boosting salt tolerance through increased Ca^{2+} and K^+ uptake. Additionally, brassinosteroids activate stress-alleviating hormones like abscisic acid, demonstrating antistress effects and interaction with other hormones to maintain ion homeostasis (Liu et al. 2014).

5.4 Senescence

Senescence in plants is a multifaceted process involving gene expression and intricate signaling pathways. Leaf senescence, a genetically orchestrated phenomenon, facilitates the transfer of nutrients from aging organs to newly emerging tissues. Governed by both developmental cues and environmental triggers, this process undergoes metabolic reprogramming. Plant hormones, such as ethylene, ABA, and brassinosteroids, contribute to the promotion of senescence, evident in the yellowing of cotyledons and leaves due to chlorophyll breakdown. Conversely, auxins, cytokinins and gibberellins act as senescence inhibitors, impeding the aging process. It serves as the final phase of plant development, allowing the retrieval of essential cellular components deposited during growth. Efficient senescence

is vital for a plant's survival and future generations. Brassinosteroids a type of plant hormone, play a crucial role in regulating senescence. Notably, brassinosteroids accelerate senescence in cucumber cotyledons and leaves of mung bean and wheat. Conversely, *Arabidopsis* mutants lacking bioactive brassinosteroids exhibit delayed senescence of chloroplasts (Clouse 2011).

5.5 Vascular differentiation

In *Arabidopsis*, BRL1 and BRL3 act as recently discovered receptors for brassinosteroids, contributing specifically to the regulation of vascular development. In *Arabidopsis*, indicating that BES1 and its homolog BZR1 redundantly promoted both phloem and xylem differentiation. However, the mechanism of BES1 in vascular development, especially its downstream components, remains largely unknown. Recent studies also revealed that brassinosteroid signaling was closely related to secondary xylem formation in trees. A reduced lignification and altered cell-wall carbohydrate biosynthesis occurred within secondary xylem of *Liriodendron tulipifera* trees through the exogenous application of brassinosteroid (Caño-Delgado et al. 2004).

5.6 Tolerance to abiotic stress

Brassinosteroids are plant hormones that play a crucial role in enhancing a plant's tolerance to abiotic stress. They help the plant respond and adapt to challenging environmental conditions, such as drought, salinity, or extreme temperatures. Brassinosteroids generally enhance a plant's ability to cope with various stresses, including drought, salinity, heat, cold, heavy metals, pesticides, and organic pollutants. Exceptions aside, these hormones contribute to improved plant adaptations in the face of diverse biotic and abiotic challenges (Kagale et al. 2007).

Applying external brassinosteroids boosts the activity of genes associated with autophagy and promotes the creation of autophagosomes in plants. When BZR1 is overexpressed, it enhances autophagosome formation and improves tolerance to nitrogen (N) starvation. Conversely, silencing BZR1 hampers autophagosome formation and reduces BR-induced tolerance to N starvation. However, the application of exogenous brassinosteroid worsens plant sensitivity to iron deficiency, indicating a dual role of BR in plant tolerance to nutrient deficiencies (Wang et al. 2012). Applying naturally occurring substances called brassinosteroids have huge impact on maize growth under drought conditions. In a rain-protected wire-house, maize underwent a 6-day drought period during tasseling, followed by foliar spray of brassinosteroid. The goal was to observe how this treatment influenced growth and gas exchange in maize, specifically assessing its ability to alleviate the negative effects of drought (Anjumet al. 2011).

6. Conclusion

Brassinosteroids play a pivotal role in enhancing plant resilience to environmental stresses. These polyhydroxylated steroidal phytohormones, crucial for plant development, growth, and productivity, engage in regulating cell division, elongation, and differentiation throughout the plant life cycle. When plants face stress, Brassinosteroids receptors on the cell surface detect Brassinosteroid, initiating phosphorylation events. This activates the central transcription factor BZR1, which, in turn, governs the transcription of Brassinosteroid -responsive genes within the nucleus, orchestrating adaptive responses to diverse biotic and abiotic challenges. There are two pathways, named the early C6-oxidation pathway and late C6-oxidation pathway, both of which would be operating in a wide variety of plants.

References

Akira S, Shozo F (1997) Studies on biosynthesis of brassinosteroids. Biosci Biotechnol Biochem 61(5): 757–762.

Alyemeni MN, Al-Quwaiz SM (2016) Effect of 28-homobrassinolide on the performance of sensitive and resistant varieties of *Vigna radiata*. Saudi J BiolSci 23(6): 698–705.

Anjum SA, Wang LC, Farooq M, Hussain M, Xue LL, Zou CM (2011) Brassinolide application improves the drought tolerance in maize through modulation of enzymatic antioxidants and leaf gas exchange. J Agron Crop sci 197: 177–185.

Caño-Delgado A, Yin Y, Yu C, Vafeados D, Mora-García S, Cheng JC, Nam KH, Li J, Chory J (2004) BRL1 and BRL3 are novel brassinosteroid receptors that function in vascular differentiation in *Arabidopsis*. Development 131: 5341–5351.

Chung Y, Choe S (2013) The regulation of brassinosteroid biosynthesis in *Arabidopsis*. Crit Rev Plant Sci 32: 396–410.

Clouse SD (2011) Brassinosteroids. *Arabidopsis* B 9: e0151.

Clouse SD, Sasse JM (1998) Brassinosteroids: essential regulators of plant growth and development. Annu Rev Plant Physiol Plant Mol Biol 49(1): 427–451.

Fridman Y, Savaldi-Goldstein S (2013) Brassinosteroids in growth control: how, when and where. Plant Sci 209: 24–31.

Fujioka S, Yokota T (2003) Biosynthesis and metabolism of brassinosteroids. Annu Rev Plant Biol 54(1): 137–164.

Kagale S, Divi UK, Krochko JE, Keller WA, Krishna P (2007) Brassinosteroid confers tolerance in *Arabidopsis thaliana* and *Brassica napus* to a range of abiotic stresses. Planta 225(2): 353–364.

Li J, Chory J (1999) Brassinosteroid actions in plants. J Exp Bot 50(332): 275–282.

Liu J, Gao H, Wang X, Zheng Q, Wang C, Wang X, Wang Q (2014) Effects of 24-epibrassinolide on plant growth, osmotic regulation and ion homeostasis of salt-stressed *Canola*. Plant Biol 16(2): 440–450.

Mandava NB (1988) Plant growth-promoting brassinosteroids. Annu Rev Plant Biol 39: 23–52.

Manghwar H, Hussain A, Ali Q, Liu F (2022) Brassinosteroids (BRs) role in plant development and coping with different stresses. Int J Mol Sci 23(3): 1012.

Müller M, Munné-Bosch S (2021) Hormonal impact on photosynthesis and photoprotection in plants. Plant Physiol 185(4): 1500–1522.

Taiz L, Zeiger E, Møller IM, Murphy A (2015) Plant physiology and Development. 6th ed. Sinauer Associates, Sunderland, CT, USA, pp 433–436.

Wang B, Li Y, Zhang WH (2012) Brassinosteroids are involved in Response of cucumber (*Cucumis sativus*) to iron deficiency. Ann Bot 110(3): 681–688.

Yu JQ, Huang LF, Hu WH Zhou YH, Mao WH, Ye SF, Nogués S (2004) A role for brassinosteroids in the regulation of photosynthesis in *Cucumis sativus*. J Exp Bot 55(399): 1135–1143.