The Multifaceted Role of Ethylene in Plant Growth

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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).

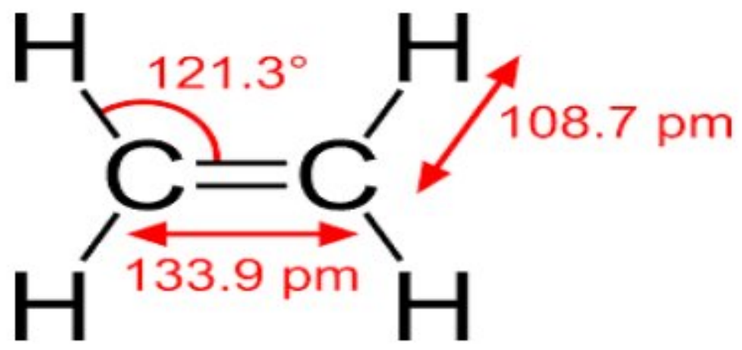

Figure 1. Structure of Ethylene

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).

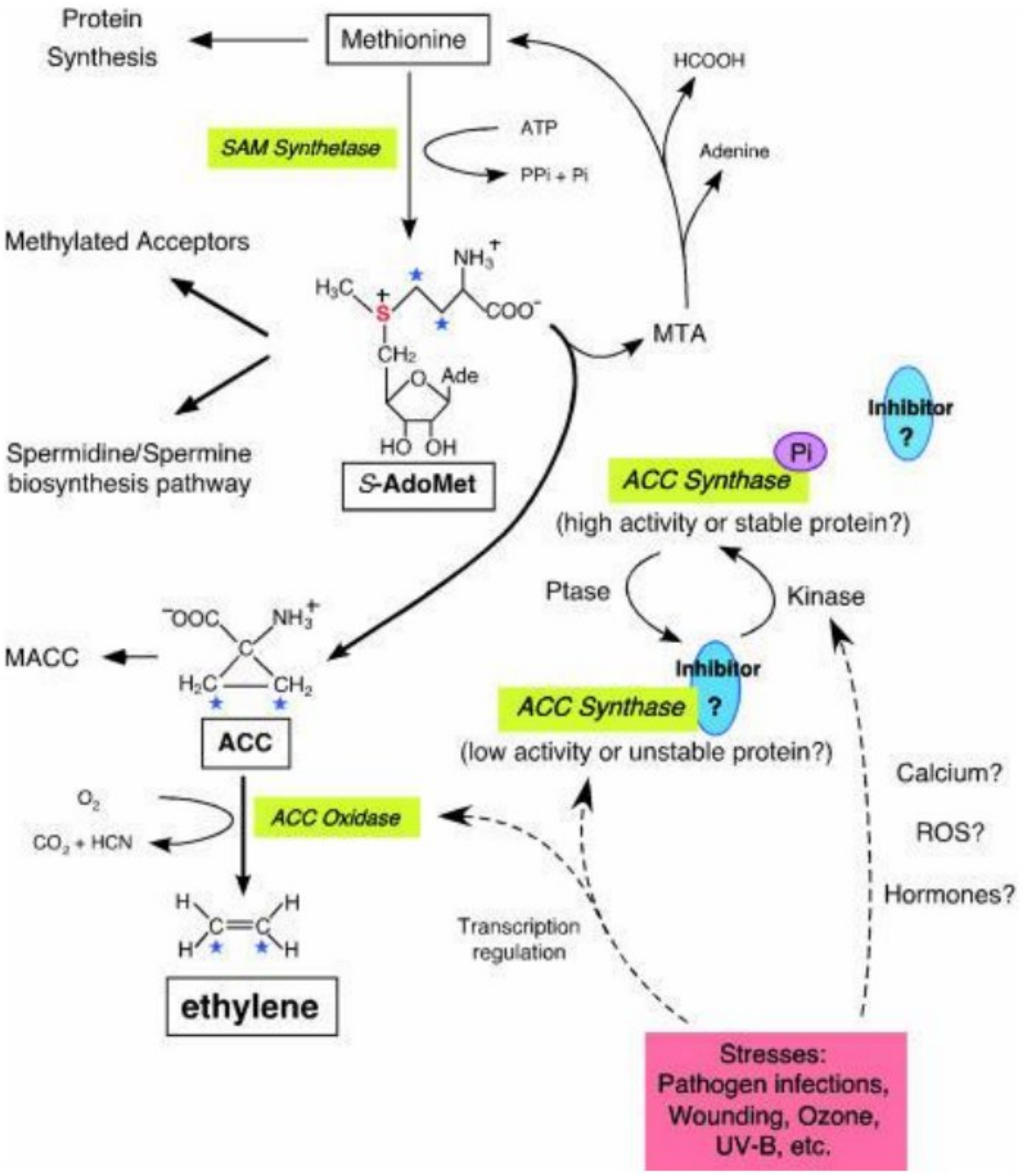

Figure 2. Biosynthetic pathway and regulation of ethylene. The formation of S-AdoMet is catalyzed by SAM synthetase from the methionine at the expense of one molecule of ATP per molecule of S-AdoMet synthesized. S-AdoMet is the methyl group donor for many cellular molecules (Methylated Acceptors), including 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.

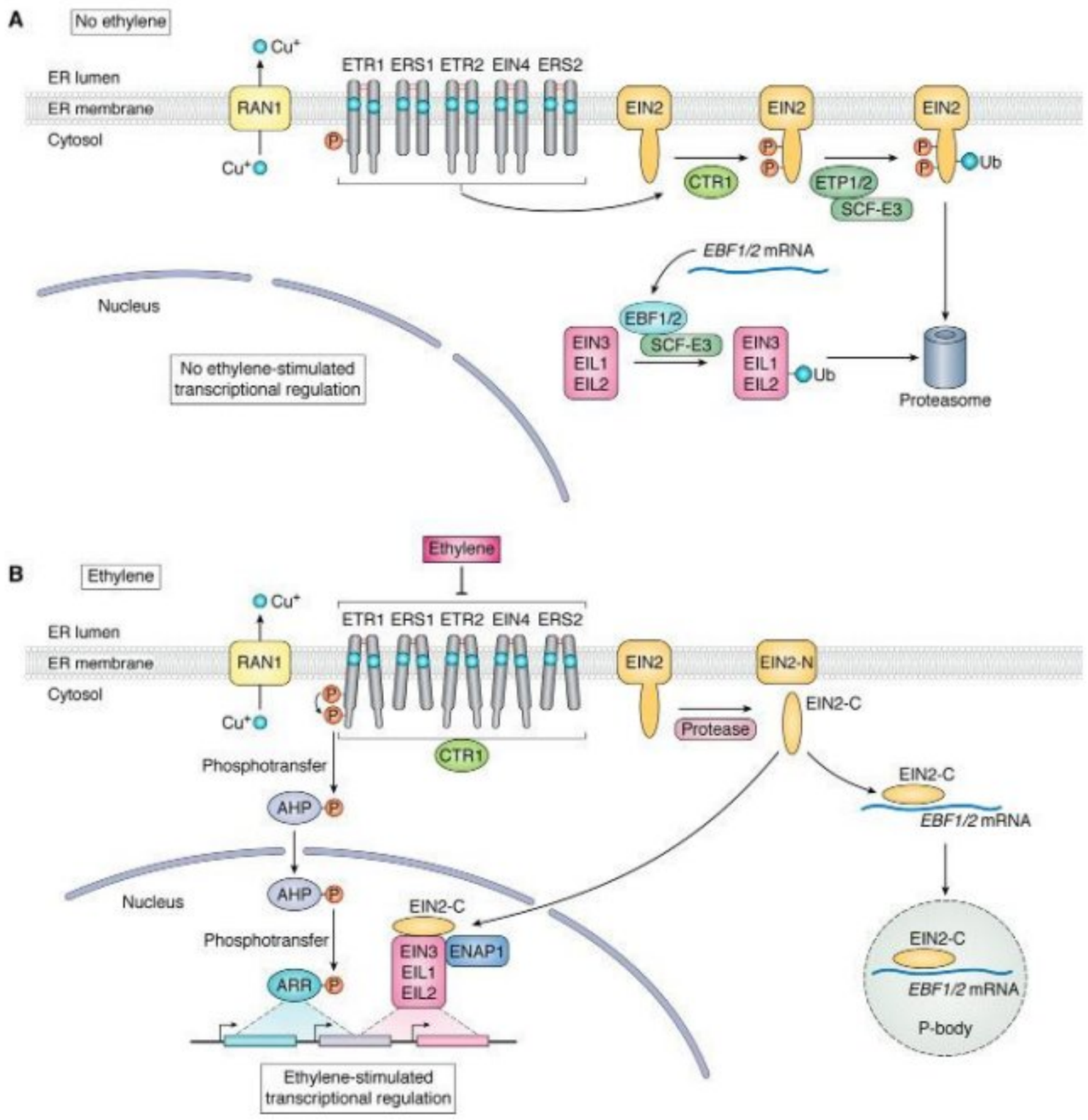

Figure 3. Model for ethylene signaling. RAN1 is a copper transporter that delivers copper to the lumen of the ER, where it is required for the biogenesis of the receptors and is used as a cofactor by the receptors to bind ethylene. A, in the absence of ethylene, the receptors signal to CTR1, which phosphorylates 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

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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).

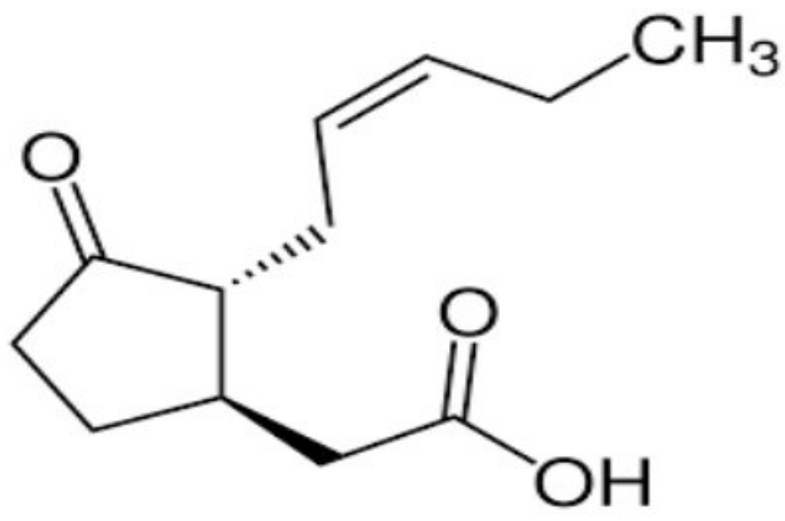

Figure 1. Structure of jasmonic acid

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).

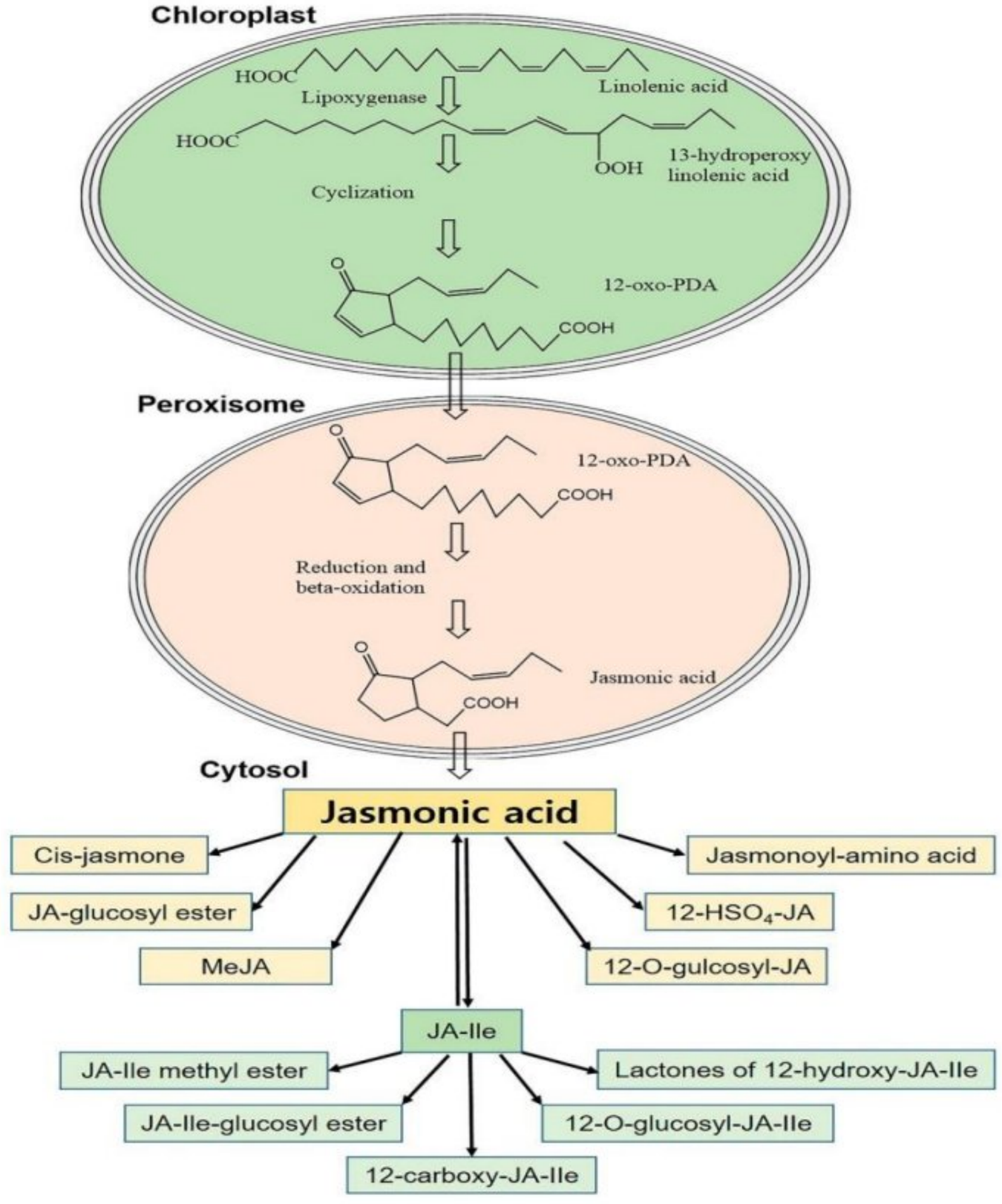

Figure 2. In chloroplast, the precursor of jasmonic acid called linolenic acid is converted into 13- hydroperoxyl linolenic acid by lipoxygenase when plant undergo any abiotic stress conditions. 13- hydroperoxyl linolenic acid undergo cyclisation and reduction it forms 12- oxo phytodienoic acid (12-OPDA). 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.