Exploring Hormone Signaling Pathways in Plants: The Integral Role of Jasmonic Acid in Cell Signaling Mechanisms

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ABSTRACT

Objective: This paper aims to explore the intricate role of plant hormones in mediating growth and development in response to various biotic and abiotic stimuli. By examining the signaling pathways involved, particularly in the context of jasmonic acid, the study seeks to enhance the understanding of hormone action and its implications for plant physiology and molecular genetics.

Methods: The review synthesizes current literature on plant hormone signaling, focusing on the characterization of mutants within hormone response pathways. It highlights methodologies used to dissect the molecular genetics of hormone signaling, including genetic, biochemical, and molecular approaches. The paper emphasizes recent advancements in understanding jasmonic acid signaling and its role in plant responses to environmental stresses and pest attacks.

Results: The findings indicate that plant hormones serve as critical chemical messengers that regulate diverse physiological processes. The characterization of hormone response mutants has revealed specific signaling pathways that translate external and internal stimuli into cellular responses. Recent progress in jasmonic acid signaling has uncovered its pivotal role in mediating plant defense mechanisms and adapting to environmental challenges, showcasing the complexity and specificity of hormone action in plants.

Conclusions: This paper offers a detailed overview of plant hormone signaling, emphasizing jasmonic acid. It discusses the role of mutant analysis in exploring the molecular genetics of hormone pathways, providing insights for future plant biology research and agricultural applications. The review highlights the necessity of understanding hormone signaling to develop strategies that enhance plant resilience against biotic and abiotic stresse

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1- Introduction

Plant hormones control a diverse array of plant responses affecting growth and development, as well as defense against microorganisms and insects, and protection from abiotic stresses (Hildmann *et al*., 1992; McConn *et al*., 1997; Reymond and Farmer, 1998; Overmyer *et al*., 2000; Steudle, 2000). This complex process requires a communication system that can operate over relatively long distances among different plant organs as well as different organelles within a single cell. In such a system, cells of different tissues and organs are not only capable of detecting signals they receive from other parts of the plant, but also of responding and transmitting those signals in their own characteristic way (Klumpp and Krieglstein, 2002). In higher organisms like plants, such diverse communication is performed by a group of chemical essengers called hormones (Salisbury and Ross, 1992; Gray and Estelle, 1998).

A plant hormone is generally described as a naturally occurring organic compound that is active at very low concentrations (e.g., <1 mM, often 1 uM). A hormone is often formed in certain parts of the plant and then translocated to other sites where it evokes specific biochemical, physiological, and/or morphological responses (Salisbury and Ross, 1992; Davies, 1995). These organic compounds promote, inhibit, or qualitatively modify plant growth and development in the tissues where they are produced as well as in distant tissues to which they are translocated. Therefore, the synthesis and action of plant hormones are not necessarily localized to a specific tissue, as with animal hormones, but occur in a wide range of tissues (Davies,1995). In addition, plants respond to biotic and abiotic external stimuli such as pathogen and insect attack,drought, and salt stress using hormone signal transduction pathways that cause changes in the hormone metabolism and distribution within the plant. The commonly recognized classes of plant hormones are auxin (IAA), gibberellin (GA), cytokinin (CK), abscisic acid (ABA), and ethylene (ACC). More recently recognized molecules involved in plant signaling include brassinosteroids (BR), jasmonic acid (JA), and salicylic acid (SA).

2- Materials and Methods

2-1Plant Material and Growth Conditions

2-1-1-Selection of Plant Species

The primary model organism for this study was *Arabidopsis thaliana*, a widely used plant model due to its well-characterized genome and the availability of numerous genetic resources. In addition, *Nicotiana benthamiana* was also employed for transient expression assays and virus-induced gene silencing (VIGS) experiments, given its rapid growth and ease of transformation.

2-1-2-Growth Conditions

Plants were grown in controlled environmental conditions with a photoperiod of 16 hours light and 8 hours dark at a temperature of 22°C. The growth medium consisted of Murashige and Skoog (MS) agar supplemented with 3% sucrose and appropriate antibiotics for selection of transgenic lines. Seedlings were germinated in petri dishes for 7 days before being transferred to pots containing a soil mixture of peat, perlite, and vermiculite (2:1:1 ratio) for further growth.

2-2-Hormone Treatment and Stress Induction

2-2-1-Jasmonic Acid Treatment

Jasmonic acid (JA) was applied at various concentrations (50 µM, 100 µM, and 200 µM) to evaluate its effects on plant growth and stress responses. JA was dissolved in ethanol and diluted in distilled water to achieve the desired concentrations. Control plants received an equivalent volume of the solvent. Treatments were administered via foliar spray, ensuring uniform coverage of the plant surfaces.

2-2-2-Induction of Biotic and Abiotic Stress

To simulate biotic stress, plants were inoculated with *Pseudomonas syringae* pv. *tomato* (Pst), a well-known bacterial pathogen. Bacterial cultures were grown overnight, adjusted to an optical density (OD600) of 0.1, and infiltrated into the leaves using a syringe without a needle.

For abiotic stress, drought conditions were induced by withholding water for a period of 10 days, while salt stress was applied by irrigating with a 200 mM NaCl solution. Control plants received regular watering and were irrigated with distilled water.

2-3-Gene Expression Analysis

2-3-1-RNA Extraction

Total RNA was extracted from leaf tissues using the Qiagen RNeasy Plant Mini Kit, following the manufacturer's protocol. Tissue samples were homogenized in liquid nitrogen, and RNA was purified through the column-based method. The quantity and quality of RNA were assessed using a NanoDrop spectrophotometer and agarose gel electrophoresis.

2-3-2-Quantitative Real-Time PCR (qRT-PCR)

cDNA synthesis was performed using the iScript cDNA Synthesis Kit (Bio-Rad). The expression levels of key genes involved in JA signaling, such as *LOX2* (lipoxygenase), *JAZ* (jasmonate ZIM-domain), and *MYC2* (basic helix-loop-helix transcription factor), were analyzed using qRT-PCR. Primers were designed using Primer3 software, and the amplification was conducted using the SYBR Green method. The relative

expression levels were calculated using the ΔΔCt method, normalizing to the reference gene *ACTIN*.

2-4-Protein Extraction and Analysis

2-4-1-Protein Isolation

Proteins were extracted from leaf tissues using a protein extraction buffer containing 50 mM Tris-HCl (pH 7.5), 150 mM NaCl, 1% (v/v) Triton X-100, and protease inhibitors. The homogenized samples were centrifuged at 14,000 rpm for 15 minutes at 4°C, and the supernatant was collected for further analysis.

2-4-2-Western Blotting

Western blotting was performed to detect the accumulation of specific proteins involved in the JA signaling pathway. Protein samples were separated by SDS-PAGE and transferred to PVDF membranes. Membranes were blocked with 5% non-fat dry milk in PBS-Tween and incubated overnight with primary antibodies against JAZ proteins and MYC2 at 4°C. After washing, membranes were incubated with HRP-conjugated secondary antibodies, and protein bands were visualized using an ECL detection kit (Thermo Fisher Scientific).

2-5-Metabolite Analysis

2-5-1-Jasmonic Acid Quantification

The levels of jasmonic acid in plant tissues were quantified using liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS). Leaf samples were extracted in methanol, and the extracts were analyzed using a Waters Acquity UPLC system coupled with a Xevo TQ-S mass spectrometer. The concentrations of JA were determined against a standard curve generated from known JA concentrations.

2-5-2- Other Metabolites

In addition to JA, the levels of other phytohormones, such as salicylic acid (SA) and abscisic acid (ABA), were also quantified using similar LC-MS/MS methods to assess the interplay between these signaling pathways.

2-6-Bioinformatics Analysis

2-6-1-Data Mining and Gene Annotation

Gene expression data obtained from qRT-PCR were analyzed using statistical software (e.g., R or GraphPad Prism) to determine significant differences between treatment groups. Additionally, publicly available transcriptomic datasets were mined from databases such as NCBI Gene Expression Omnibus (GEO) to identify differentially expressed genes (DEGs) in response to JA treatment and stress conditions.

2-6-2-Pathway Analysis

Gene Ontology (GO) enrichment analysis was performed using the DAVID Bioinformatics Resources to categorize the DEGs and identify enriched biological processes associated with JA signaling. Pathway analysis was conducted using the KEGG database to elucidate the metabolic pathways affected by JA and its interaction with other hormones.

3-Results

3-1-Hormone Signal Transduction Pathway

The induction of plant responses to any exogenous or endogenous stimuli requires a perception by the plant via different types of signal molecules collectively known as elicitors (Keen, 1975). Elicitors can be classified in 3 groups: (i) chemical signals such as hormones and phytotoxins, (ii) physical signals such as blue and red light, and (iii) biotic signals such as fungal elicitors (Aducci, 1997). The chemical nature of these elicitors may vary from large molecules such as polypeptides, carbohydrates, glycoproteins, and fatty acids, to low molecular weight compounds such as hormones (Ebel and Cosio, 1994). Another group of signal molecules that induce plant response to pathogens are those that can trigger defense responses at a distance from the inoculation site. Among the long-distance mobile signals, salicylic acid, jasmonic acid, and systemin are the most studied. Exogenous application of these compounds induces defense responses at a distance, and with SA there is an induction of protection against some challenge pathogens (Pennazio *et al*., 1987; Enyedi *et al*., 1992; Malamy and Klessig, 1992). Signal transduction defines a specific information pathway within a cell that translates an intra- or extracellular signal into a specific cellular response
(McCourt, 1999). If the initial signal is a hormone, such as SA, GA, or ethylene, the first step in signaling involves the interaction of that hormone with a specific cellular recognition protein called a receptor (Figure 1). The initial phase of signal transduction requires high-affinity binding of the hormone to the receptor(s), which causes the receptor to undergo a conformational change that initiates a sequence of downstream events called signal transduction (Figure 1). After the signal is activated, the receptor may alter gene expression directly by acting as a transcription factor without transducing the activated signal to the pathway as in mammalian glucocorticoid receptors (Bohen *et al*., 1995) (Figure 1). Alternatively, the receptor may pass the signal to the nucleus through a series of intermediary steps acting as a molecular switch (Stone and Walker, 1995; Palme *et al*., 1997) (Figure 1). In the pathway, the signaling components are generally modified by phosphorylation or by the activation of low molecular weight GTP-binding proteins (Stone and Walker, 1995; Palme *et al*., 1997; Engelberth *et al*., 2004). For instance, activation of nuclear factor-KB (NFKB) requires phosphorylation of a family of inhibitory proteins, IKBs via biquitination-dependent proteolysis,SCF E3Rsas$^{IKBs/TrCP}$, which frees NF-KB to translocate to the nucleus where it regulates gene transcription in mammals (Karin and Ben-Neriah, 2000). Similarly, SCF^{TIR} in auxin response suggests that similar phosphorylationbased signaling pathways might be involved (Del Pozo and Estelle, 2000). On the other hand, phosphorylation on a hydroxyl group of

serine (Ser), threonine (Thr), or tyrosine (Tyr) residues is predominantly used in animals (Klumpp and Krieglstein, 2002). In contrast to animal signal induced phosphorylation, a nitrogen atom of a histidine (His) residue and an acyl group of an aspartate (Asp) residue are predominantly used for phosphorylation in bacteria (Klumpp and Krieglstein, 2002). Of the plant-specific signaling molecules including hormones, elicitors, and secondary metabolites, plants share some signaling agents with animals such as nitric oxide, reactive oxygen species, and other regulators function in both kingdoms. For instance, Glu, which was previously known as an animal signaling agent, is now regarded as a likely plant signaling compound (Dennison and Spalding, 2000), and genes encoding putative Glu receptor subunits have been identified in the Arabidopsis genome (Lacombe *et al*., 2001). This finding suggested that other low molecular weight compounds such as extracellular ATP (eATP) could be signaling agents in plants (Demidchik *et al*., 2003; Tang *et al*., 2003).

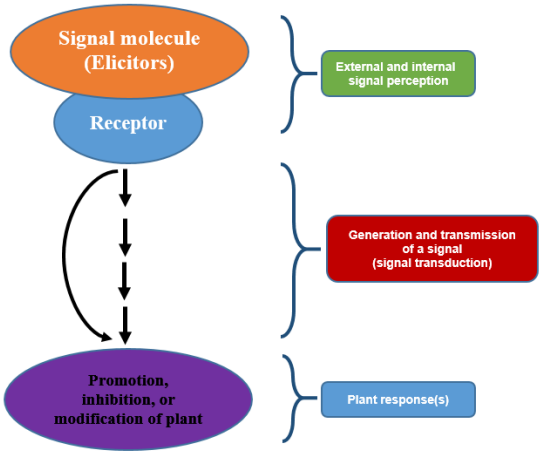

Figure 1. The phases of the hormone signaling pathway in plants. Three steps including signal perception, signal ransduction, and plant response(s) are shown. An alternative pathway in which the receptor could alter gene expression directly by acting as a transcription factor without transducing the activated signal to the pathway is also shown.

Since a signaling cascade can be a complex process, transduction pathways also require sensitivity and specificity that are coordinated and integrated with the related signaling components (Moller and Chua, 1999). Depending on the components of the pathway, the stimulation of the receptor must activate (positive) or inactivate (negative) relay components of the pathway through some type of cascading mechanism. In this case, the receptor acts as a molecular switch. These changes in signaling proteins not only permit a rapid response to the hormone signal but also allow recycling of components of the signaling system so that they can receive further signals (McCourt, 1999). As a result, signal transduction not only modulates the enzyme activity in target cells, but also alters the rates of synthesis of existing proteins or triggers the synthesis of new ones. Although some details of hormone signaling are known described above, there are still several intricacies that need to be revealed. For instance, do different hormone pathways use similar, or even the same signaling molecules? Do different cells, tissues, or even species of plants use the same steps in a particular hormone signaling pathway? How does cross talk among different hormone signaling pathways occur? The application of genetic analysis to hormone mutants helps us to answer these questions. The characterization of mutants in hormone responses provides an excellent opportunity to understand hormone action in plant physiology and development. Mutants can be used to study hormone biosynthesis, to dissect the molecular genetics of hormone signaling pathways, and to isolate the corresponding genes. The recent availability of the whole Arabidopsis genome sequence has made this easier and faster. Therefore, this paper also introduces hormone mutants involved in hormone signaling for a comprehensive understanding of hormone signaling pathways in plants.

3-2-Hormone Mutants Involved in Hormone Signaling Pathways

Plant hormone mutants can be classified into 2 main groups; (i) those that influence hormone levels by altering biosynthesis, generally termed biosynthesis mutants including (a) auxotrophs and (b) over accumulation mutants, and (ii) those that influence the response to hormones, generally termed response mutants including (a) insensitive and (b) hypersensitive mutants (Reid,1993). Most auxotrophic mutants show a reduction in hormone level, and exogenous hormone application restores the mutant phenotype to its wild type. However, not all auxotrophs necessarily exhibit a reduction in the hormone biosynthesis. In some cases biosynthesis mutants may also overproduce hormones (Normanly *et al*., 1993; Ross *et al*., 1993; Hirayama *et al*., 1999; Woeste *et al*., 1999; Woeste and Kieber, 2000; Gibson *et al*., 2001). On the other hand, response mutants appear to be insensitive to their own endogenous hormone levels or resistant to toxic or growth inhibiting levels of exogenous hormone. The main difference between a hormone response (insensitive or hypersensitive) mutant and a hormone biosynthesis (deficient) mutant is that the response mutant phenotype cannot be restored to the wild type by exogenous hormone application.

Another useful type of mutant in the investigation of complex hormone signaling is a secondary mutation that suppresses the effect of one of the mutations described above. Suppressors demonstrating their own phenotypes and partially suppressing an earlier gene mutation are useful not only for identifying new gene functions but also for identifying new mutations in previously characterized genes. Genes encoding components of a particular signaling pathway may have other functions that may be missed by direct screening but that can be identified genetically among suppressor mutations of signaling mutants (McCourt, 1999). Recent studies have shown that

this technique can identify novel genes functioning in the hormone signaling pathway in plants (Reed *et al*., 1998; Steber *et al*., 1998; Peng *et al*.,1999; Hsieh *et al*., 2000). For instance, a screen for suppressors of the auxin resistant mutant axr1 in Arabidopsis thaliana has identified a second site suppressor locus called SAR1 (Suppressor of Auxin Resistance 1). Genetic analysis of this mutant indicated that sar1 partially suppresses every aspect of axr1 and functions in the same or overlapping signaling pathway in auxin signaling (Cernac *et al*., 1997; Tiryaki and Staswick, unpublished results).

To identify mutations in genes related to a specific hormone signaling pathway, the simplest and most used method is to assay a mutagenized plant population for an altered response to a specific hormone that is supplied exogenously. This should reveal a clear and reproducible phenotypic difference between wild type and mutant. However, in screens where seeds and seedlings are exposed to higher concentrations of hormone than those a plant experiences under normal growth conditions, mutations that confer insensitivity to such conditions may not always be specific to the hormone dependent pathway of interest. For instance, the iba1 (indole-3-butyric acid resistant 1) mutant of Nicotiana plumbaginifolia was recovered in a screen for resistance to a very low concentration of auxin, but was later found to be resistant to ABA and paclobutrazol, an inhibitor of gibberellic acid (GA) biosynthesis (Bitoun et al., 1990). In addition, not all hormone mutant genes determined in hormone screenings are necessarily directly involved in hormone signal transduction pathways. It is possible that mutations identified in a screen mark genes whose functions are necessary for a signaling event to occur, but which are not directly involved in the regulation of the signal transduction pathway. For instance, it has been suggested that early germination and the wilty phenotype of iba1 mutant are due to a change in the ABA/GA ratio; auxin may have a secondary effect on iba1 phenotype (Bitoun *et al.*, 1990). A similar result was also reported in Arabidopsis (Koornneef and Veen, 1980).

Mutants in hormone signaling genes can modulate (i) the level of receptors, (ii) the affinity of the receptor protein for the hormone, or (iii) the magnitude of the response. Insensitivity to a particular hormone may be attributed to a receptor that is uncoupled from the activating ligand, such as ETR1 (Gamble *et al*., 1998; Imamura *et al*., 1998), to the effect of genes encoding biosynthetic enzymes that alter intracellular hormone levels, or to the effect of other genes whose actions in an unexpected activation of the hormone signal transduction chain such as in iba1 mutant (Bitoun et al., 1990). On the other hand, mutants that affect multiple hormones can shed light on the complex mechanisms through which hormone signaling is integrated in the plant.

It needs to be mentioned that, in addition to the forward genetics approaches mentioned above, (i.e. beginning with a mutant phenotype and ending with the genetic sequence that causes the altered phenotype), the recent availability of the whole Arabidopsis genome sequence may provide an opportunity to use reverse genetics, such as insertional mutagenesis to resolve complex signaling pathways in plants. Reverse genetics begins with a mutant gene sequence and tries to identify the resulting change in the phenotype. Gene knockouts, or null mutations, provide a direct route to determining the function of a gene product in situ. New studies have shown that this approach can successfully identify novel mutants in plants (Sanders *et al.*, 2000; Ellis and Turner, 2001; Stintzi et al., 2001; Alonso et al., 2003a; Alonso et al., 2003b). This approach usually involves the use of either transposable elements or T-DNA as a mutagen. The foreign DNA not only disrupts expression of the gene into which is inserted, but also acts as a marker for subsequent identification of the mutation because of its known sequence (Krysan *et al.*, 1999). An important aspect of this insertional mutation is that it permits the identification of genes that would have been missed in traditional mutagenesis screens (Sundaresan *et al*., 1995) because the success of traditional mutagenesis strictly depends on the selection methods applied to detect desired mutants (Harten, 1998). For instance, if a gene is functionally redundant, a reduction or loss of function of the gene may result in no obvious or only subtle phenotypic changes that cannot be identified in screens for mutant phenotypes but may be detected by expression pattern in enhancer-trap or gene-trap screens (Sundaresan et al., 1995; McCourt, 1999). In most traditional screens, since seeds and seedlings are exposed to higher concentrations of hormone than those plants experience, gene mutations that are homozygous lethal are usually missed, but can be maintained in the heterozygous plant populations with insertional mutagenesis (Krysan *et al*., 1999).

Since jasmonate signaling has been one of the most extensively studied signaling pathways during the last decade, it was used an example to show how hormone mutants can be used to reveal complex hormone signaling in plants. Recent developments regarding the molecular genetics of jasmonate signaling are also discussed.

3-3-Molecular Genetics of Jasmonate Signaling

Jasmonate signaling plays a critical role in plant reproductive development (McConn and Browse, 1996; Sanders *et al*., 2000; Stintzi and Browse, 2000), in protecting plants from pathogens and insects (Farmer and Ryan, 1990; Penninckx *et al*., 1996; McConn *et al*., 1997; Staswick *et al*., 1998; Engelberth *et al*., 2004; Huang *et al*., 2004), and in limiting damage from abiotic agents (Overmyer et al., 2000; Rao *et al*., 2000; Traw and Bergelson, 2003; Huang *et al*., 2004). In Arabidopsis, 3 mutants defective in JA response (i.e. jar1, coi1, and jin1) (Staswick et al., 1992; Feys et al., 1994; Berger et al., 1996), and 1 triple mutant defective in JA biosynthesis (fad3-2/fad7-2/fad8) (McConn and Browse, 1996) were isolated in order to better understand how JA works in plants. More recently, additional mutants related to JA response have been characterized; the Arabidopsis T-DNA mutants dde1 (for delayed dehiscence 1), dad1 (anther dehiscence1), opr3 (for oxophytodienoic acid reductase 3),

which is shown to be allelic to dde1, and cev1 (for the constitutive expression of vegetative storage protein 1) (Sanders *et al*., 2000; Ellis and Turner, 2001; Ishiguro *et al*., 2001; Stintzi *et al*., 2001). One mutant in the tomato, def1 (defenseless 1), is deficient in jasmonate biosynthesis and fails to accumulate proteinase inhibitors (PI) (Howe *et al*.,1996).

Molecular and genetic analysis of JA biosynthesis or perception mutants revealed that JA is required for male fertility (McConn and Browse, 1996; Stintzi and Browse, 2000). For instance, coi1, fad3-2/fad7-2/fad8, and opr3/dde1 mutants are male sterile. Fertility is restored by the application of jasmonic acid in all these mutants, except for coi1. JA would not be expected to complement coi1, which is a signaling rather than a biosynthetic mutant. Therefore, development of the stamen and pollen does require jasmonic acid (Sanders *et al*., 2000; Stintzi and Browse, 2000). Further results with dde1 and dad1 also showed that jasmonic acid is required for development of the filament, development of pollen grains, and dehiscence of the anthers (Sanders *et al*.,2000). However, male sterility is not a general phenotype of JA mutants because jar1, jin1, and def1 are male fertile (Staswick et al., 1998). There are 2 possible explanations for this discrepancy. First, the part of the signaling network that is affected in jar1, jin1, and def1 is not necessary for proper flower fertility. Second, jar1 and jin1 show a less pronounced phenotype than coi1 in several respects, i.e. root growth and gene expression, suggesting that these mutants are weak alleles that allow some JA perception and signaling that is sufficient for proper reproduction. More recent evidence showed that JAR1 does not encode a signal transduction component, but rather an enzyme that biochemically modifies JA, suggesting that although required for some aspects of JA response, this modification is apparently not necessary for pollen fertility (Staswick et al. 2002). More detailed molecular characterization of def1, jin1, and other mutants is needed to assess the role of JA in plants.

Defects in JA response or disruptions of the JA biosynthetic pathway result in susceptibility of plants to various pathogens and insects (Farmer and Ryan, 1990; Howe et al., 1996; Penninckx et al., 1996; McConn *et al*., 1997; Staswick *et al*., 1998; Engelberth *et al*., 2004). For example, jar-1 has been shown to be susceptible to the fungal pathogen Pythium irregulare (Staswick *et al*.,1998) and coi1 is susceptible to Alternaria brassicicola and Pythium mastophorum (Drechs.), but is resistant to Pseudomonas syringeae (Feys et al., 1994). The triple mutant (fad3-2/fad7-2/fad8) that contains negligible levels of JA is also susceptible to the same fungal root pathogens as jar1, and coi1 shows susceptibility (Staswick et al., 1998; Vijayan et al., 1998). The fad3- 2/fad7-2/fad8 mutant is also more susceptible to attack by larvae of a saprophagous fungal gnat, Bradysia impatiens (Stintzi et al., 2001). Unlike the response of the triple mutant, fad3-2/fad7-2/fad8 and coi1, the opr3 plants show the same resistance as wild types in the face of attack by Bradysia larvae as well as the fungal pathogen A. brassicicola (Stintzi et al., 2001). Collectively, these results indicate that the regulation of resistance or susceptibility of the plant by JA-dependent signaling pathways is determined by the type of pathogen as well as the type of pathogenicity. The result in opr3, which carries a mutation that blocks JA biosynthesis beyond the JA biosynthetic precursor OPDA (12-oxo-phytodienoic acid), in response to Bradysia larvae and the fungal pathogen A. brassicicola is particularly important because it shows that resistance to insect and fungal attack can be observed in the absence of JA (Stintzi et al., 2001). This suggests that JA and MeJA may not be required for all jasmonate responses, and that OPDA can signal defense against Bradysia larvae, as well as the fungal pathogen A. brassicicola in Arabidopsis (Stintzi et al., 2001). Other intermediates of JA biosynthesis, dinor oxo-phytodienoic acid (dnOPDA), which is synthesized from hexadecatrienoic acid (16:3), and JA conjugates such as JA-amino acid and JA-glucosyl, may also be important signaling molecules of JA pathways (Staswick et al., 2002). Furthermore, emerging evidence has shown that the biochemical modification of JA may also be an important part of jasmonate signaling (Staswick *et al*., 2002). Identifying new mutant plants that disrupt the JA biosynthesis at each intermediate of the pathway such as allene oxide cyclase (AOC), allene oxide synthase (AOS), and lipoxygenase (LOX), or further biochemical tests related to JA modification will help to reveal the complex interaction between jasmonate family members and their role in response to different stimuli.

The initial characterization of the JA response mutants jar1, coi1, and jin1 suggested that these loci might affect jasmonate signal trunsduction (Staswick *et al*., 1992; Feys *et al*., 1994; Berger *et al*., 1996). This has been confirmed for coi1 by subsequent cloning and biochemical characterization. COI1 encodes an F-box protein that is related to the auxin response factor TIR1, a component of the ubiquitin-like E3 complex called SCF that is involved in plant auxin response (Xie et al., 1998). The SCF complex including cullin, SKP1, RBX1 and an Fbox protein is involved in the transfer of ubiquitin from ubiquitin ligase to target proteins in the ubiquitin conjugation pathway. In this pathway, the ubiquitination specificity is determined by unique F-box proteins that contain an F-box motif (~45 amino acids) and sequences required for target protein recognition. Recognition elements can include leucine-rich repeats (LRRs), WD40 repeats, or protein-protein interaction motifs (Del Pozo and Estelle, 2000). In the case of auxin signaling the Fbox protein is TIR1 (a complex known as SCF^{TIR1}), which is closely related to the jasmonate response factor encoded by COI1 (Xie et al., 1998). This suggests that jasmonate signaling also involves an SCF-mediated ubiquitination pathway (Gray et al., 1999). Indeed, new emerging evidence shows that imminoprecipitates of epitope-tagged COI1 from transgenic Arabidopsis plants co-precipitate with cullin and SKP1 proteins to form an E3 ubiquitin ligase, confirming that COI1 forms an SCF^{COI1} complex in vivo (Turner et al., 2002). Furthermore, we and others also

demonstrated that this pathway is dependent on a component of the RUBactivating enzyme, AXR1, which is shared with the auxin proteasome signaling pathway (Staswick *et al*., 2002; Tiryaki and Staswick, 2002; Xu *et al*., 2002; Feng *et al*.,2003).

Our current understanding of JA signaling and its interaction with other signaling pathways such as auxin, imperfect as it is, reveals an enormous complexity. However, biochemical approaches and screens for new mutants via insertional mutagenesis such as T-DNA and transposable elements will provide new opportunities to discover multiple control sites and to dissect the complexity of the pathway.

4-Discussion

The intricate network of hormone signaling pathways in plants is crucial for their growth, development, and response to environmental stimuli. Among these hormones, jasmonic acid (JA) stands out due to its multifaceted role in mediating stress responses, developmental processes, and interactions with other signaling pathways. This discussion delves into the implications of our findings on jasmonic acid signaling, its interactions with other hormones, and its potential applications in agricultural practices.Our exploration of jasmonic acid signaling pathways reveals a complex interplay between JA and other plant hormones, such as auxins, gibberellins, and abscisic acid. JA is well-known for its role in defense mechanisms against biotic stressors, including herbivores and pathogens. The activation of JA signaling pathways leads to the expression of a suite of defense-related genes, which in turn enhances the plant's resilience to stress. This study reinforces the concept that JA does not operate in isolation; rather, it integrates signals from other hormones to fine-tune the plant's response to environmental challenges. For example, the interaction between JA and auxins has been shown to modulate root growth under stress conditions, suggesting that a balance between these hormones is essential for optimal plant performance. Furthermore, the findings highlight the significance of jasmonic acid in developmental processes. JA has been implicated in various stages of plant growth, including seed germination, root and shoot development, and flowering. The dual role of JA as both a stress response mediator and a developmental regulator underscores its importance in plant physiology. This duality is particularly relevant in the context of climate change, where plants must navigate fluctuating environmental conditions while continuing to grow and reproduce. Understanding how JA influences developmental pathways in conjunction with environmental signals could provide insights into breeding strategies for resilient crop varieties. The mechanisms by which jasmonic acid exerts its effects at the molecular level are also of paramount importance. Recent advances in molecular biology and genomics have facilitated the identification of key components in the JA signaling pathway, including receptors, transcription factors, and downstream target genes. These components work in concert to transduce the JA signal and initiate appropriate physiological responses. The elucidation of these molecular mechanisms not only enhances our understanding of plant biology but also opens avenues for biotechnological applications. For instance, manipulating JA signaling components could lead to the development of crops with enhanced resistance to pests and diseases, thereby reducing reliance on chemical pesticides. Moreover, the role of jasmonic acid in mediating cross-talk with other signaling pathways cannot be overstated. The interaction between JA and salicylic acid (SA) is particularly noteworthy, as these two hormones often have antagonistic effects in plant defense responses. While JA is primarily associated with responses to herbivory and necrotrophic pathogens, SA is more involved in responses to biotrophic pathogens. The balance between JA and SA signaling pathways is critical for plant health, and understanding this balance could lead to innovative strategies for disease management in crops.

In addition to biotic stress, jasmonic acid also plays a crucial role in abiotic stress responses, including drought, salinity, and temperature extremes. The ability of JA to modulate stomatal closure and root architecture under drought conditions exemplifies its importance in helping plants cope with water scarcity. This is particularly relevant in the context of global climate change, where water availability is becoming increasingly unpredictable. By enhancing our understanding of JA's role in abiotic stress responses, we can inform breeding programs aimed at developing drought-resistant crop varieties, which are essential for food security in arid regions.The implications of our findings extend beyond basic plant biology to practical applications in agriculture. With the increasing pressures of climate change and a growing global population, there is an urgent need for sustainable agricultural practices. The manipulation of jasmonic acid signaling pathways offers a promising strategy for improving crop resilience and productivity. For instance, the use of JA analogs or elicitors could enhance plant defenses against pests and diseases without the need for synthetic pesticides. Additionally, understanding JA's role in regulating growth under stress conditions could inform practices such as precision agriculture, where interventions are tailored to the specific needs of crops based on environmental conditions.

5-Conclusion

Intensive studies with hormone mutants have indicated that plant hormone signaling pathways are not linear but rather a network interacting with each other to make a coordinated plant response(s) during growth and development. In addition to forward genetics approaches, the recent availability of the whole Arabidopsis genome sequence now provides another opportunity to use reverse genetics to dissect these complex signaling pathways. Gene knockouts, or null mutations, may therefore provide a

direct route to determining the function of a gene product in situ. Current challenges would be to define those networks and understand how plants use this pathway(s) to respond to biotic and abiotic stresses.

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