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CHARACTERIZATION OF A NEW JASMONATE SIGNALING MUTANT IN *ARABIDOPSIS*

by

Iskender Tiryaki

A DISSERTATION

Presented to the Faculty of

The Graduate College at the University of Nebraska

In Partial Fulfillment of Requirements

For the Degree of Doctor of Philosophy

Major: Agronomy

(Plant Breeding and Genetics)

Under the Supervision of Professor Paul E. Staswick

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DISSERTATION TITLE

Characterization of a New Jasmonate Signaling Mutant

in Arabidopsis

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Iskender Tiryaki

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GRADUATE COLLEGE
UNIVERSITY OF NEBRASKA

CHARACTERIZATON OF A NEW JASMONATE SIGNALING MUTANT IN *ARABIDOPSIS*

Iskender Tiryaki, Ph.D.

University of Nebraska, 2002

Advisor: *Paul E. Staswick*

Jasmonic acid and its methyl ester form, MeJA, influence several important aspects of plant growth and development, including root and shoot growth, flower fertility, senescence, to protection of plants from biotic and abiotic stresses. A screen for methyl jasmonate (MeJA) insensitive root growth in *Arabidopsis thaliana* yielded alleles of the previously isolated mutant loci *jar1* and *coi1*, with one exception, a mutant locus called *54-1*. The response of wild type and *54-1* primary root growth was tested on a range of concentrations of indole-3-acetic acid, 1-aminocyclopropane-1- carboxylic acid, 6-benzylamino-purine, epi-brassinolide, and absisic acid. The data showed that *54-1* is not only insensitive to MeJA, but also to the foregoing hormones, as well as certain other compounds that inhibit root elongation. Genomic mapping, morphological characterization, and non-complementation in a F_1 cross of *54-1* by *axr1* (*auxin resistant 1*), confirmed that *54-1* and *axr1* are the same genes. The new *axr1* allele, named *axr1-24,* was further shown to be susceptible to the opportunistic pathogen *Pythium irregulare,* a trait previously found in jasmonate response mutants. The *jar1/axr1* double mutant was more resistant to inhibition of root growth by MeJA and more susceptible to *P. irergulare* infection than either single

mutant, suggesting they define independent signaling pathways. Gene expression analysis showed that IAA induced the JA responsive genes and this induction was reduced in *axr1*. However, transcript induction by MeJA, was only minimally affected in *axr1-24*. A screen for suppressor of *axr1-24* yielded seven plants harvested from independent parental group seed pools. The new mutations in these seven lines partially suppressed most aspects of the *axr1* phenotype, including plant height, silique shape and size, fertility. Furthermore, a detailed characterization of one of these suppressor lines, *16-1*, revealed that it partially overcomes the defects in jasmonate responses, including root growth, pathogen susceptibility, and possible male infertility. These results provide new information about auxin and jasmonate response of *AXR1*, and the possible interaction between these two important signaling pathways.

DEDICATION

To

My parents Satılmış and Belgüzar TIRYAKI

&

The People of the Republic of TURKEY

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CHAPTER I

GENERAL INTRODUCTION TO PLANT HORMONES, HORMONE SIGNALING, HORMONE MUTANTS, JASMONIC ACID, and AUXIN in PLANTS

INTRODUCTION TO PLANT HORMONES

Plant growth and metabolism can be rapidly controlled by stimuli such as light, hormones and environmental stresses. 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 which they receive from other parts of the plant, but they are also responding and transmitting those signals in their own characteristic way (Libbenga and Mennes, 1995). In higher organisms like plants, such a diverse communication is done by a group of chemical messengers called hormones (Gray and Estelle, 1998; Salisbury and Ross, 1992).

A plant hormone (or phytohormone) is generally described as a naturally occurring organic compound that is active at very low concentrations (e.g., <1mM, often 1uM). 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 (Davies, 1995; Salisbury and Ross, 1992). These organic compounds promote, inhibit, or qualitatively modify plant growth and development in tissues where they are produced as well as in distant tissues to which they are translocated. Therefore, 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 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, and salicylic acid.

INTRODUCTION TO PLANT HORMONE SIGNALING

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 three 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 groups 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 (SA), 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 (Enyedi et al., 1992; Malamy and Klessig, 1992; Pennazio et al., 1987).

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 interaction of that hormone with a specific cellular recognition protein called a receptor. The initial phase of signal transduction requires high-affinity binding of the hormone to the receptor, which causes the receptor to undergo a conformational change that initiates a sequence of downstream events (signal transduction). After the signal is activated, the receptor could alter gene expression directly by acting as a transcription factor without transducing the activated signal to the pathway as in mammalian glucocorticoid receptor (Bohen et al., 1995). Alternatively, the receptor may pass the signal to the nucleus through a series of intermediary steps acting as a molecular switch (Palme et al., 1997; Stone and Walker, 1995). In the pathway, the signaling components are generally modified by phosphorylation or by the binding and hydrolysis of a guanine nucleotide (Palme et al., 1997; Stone and Walker, 1995). For instance, activation of NF-κB (nuclear factor-κB) requires phosphorylation of a family of inhibitory proteins, IκBs via ubiquitination-dependent proteolysis, SCF E3Rsas$^{I\kappa Bs/TrCP}$, which frees NF-κB 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 a similar phosphorylation-based signaling pathways might be involved (del Pozo and Estelle, 2000).

Since a signaling cascade can be a complex process, transduction pathways also require sensitivity and specificity that are coordinated and integrated with 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 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 rates of synthesis of existing proteins or triggers the synthesis of new ones.

PLANT HORMONE MUTANTS

The characterization of mutants in hormone responses provides a great 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 effort easier and faster, at least for this model plant.

Plant hormone mutants can be classified into two main groups; (i) those that influence hormone levels by altering the 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 have a reduction in the biosynthesis of hormone. Biosynthesis mutants may also overproduce (Ross et al., 1993). On the other hand, response mutants appear to be insensitive to their own endogenous levels of hormone 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 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 just 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 can be identified genetically among suppressor mutations of signaling mutants (McCourt, 1999). Recent studies showed that this technique can identify novel genes functioning in the hormone signaling pathway in plants (Hsieh et al., 2000; Peng et al., 1999; Reed et al., 1998; Steber et al., 1998). 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).

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 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 very low concentration of auxin, but was later found to be resistant to ABA and paclobutrazol, an inhibitor of giberellic 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 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