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Review

# The power of mutants for investigating jasmonate biosynthesis and signaling

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ABSTRACT

Mutant analysis includes approaches that range from traditional screening of mutant populations (forward genetics), to identifying mutations in known genes (reverse genetics), to examining the effects of site-specific mutations that encode modified proteins. All these methodologies have been applied to study jasmonate synthesis and signaling, and their use has led to important discoveries. The *fad3 fad7 fad8* mutant of *Arabidopsis*, and other mutants defective in jasmonate synthesis, revealed the roles of jasmonate in flower development and plant defense against necrotrophic fungal pathogens. The *coi1* mutant identified the F-box protein that is now known to be the receptor for jasmonoyl-isoleucine, the active form of jasmonate hormone. Investigations of how JASMONATE-ZIM DOMAIN (JAZ) proteins bind to COI1 and facilitate jasmonate perception have relied on the *jai3* mutant, on *JAZΔJas* constructs, and on site-specific mutations in the Jas and ZIM domains of these proteins.



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## 1. Introduction

Jasmonate (JA) helps to regulate diverse aspects of plant biology, ranging from stress responses, to development. JA-mediated stress responses include defense against insects and microbial pathogens as well as responses to drought, ultraviolet radiation, ozone, and other abiotic stresses (Browse, 2005; Conconi et al., 1996; Glazebrook, 2005; Howe and Jander, 2008). In healthy, unwounded tissue, JA is involved in carbon partitioning, mechanotransduction, senescence and reproductive development (Buchanan-Wollaston et al., 2005; Creelman and Mullet, 1997; Feys et al., 1994; Li, 2004). JA-dependent responses are associated

*Abbreviations: acx*, acyl-CoA oxidase; *aim1*, abnormal inflorescence meristem1; *aos*, allene oxide synthase; *ASK*, *Arabidopsis* skip1 homologue; *AXR1*, auxin-resistant1; *cas*, constitutive allene oxide synthase; *cet*, constitutive expression of thionin; *cev*, constitutive expression of vegetative storage protein; *cex*, constitutive expression; *coi1*, coranatine-insensitive1; *cos*, coi1 suppressor; *COP9*, constitutively photomorphogenic1; *CUL1*, cullin1; *dad1*, defective anther dehiscence1; *dde1*, delayed dehiscence1; *def1*, defenceless1; *dgl*, dongle; *fad*, fatty acid desaturation; *jai*, jasmonate insensitive; *jar1*, jasmonate resistant1; *jaz*, jasmonate-zim domain; *jin1*, jasmonateinsensitive1; *joe*, jasmonate overexpressing; *myb*, myeloblastosis; *opcl1*, OPC-8:0 CoA ligase1; *opr3*, OPDA reductase3; *PDF1.2*, plant defnesin1.2; *RBX1*, RING_box1; *SGT*, homologue of yeast suppressor of G2 allele of SKIP1.

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with large-scale reprogramming of gene expression and hundreds of downstream JA-regulated and -coregulated genes have been identified (Mandaokar et al., 2006; McGrath et al., 2005; Reymond et al., 2004).

In this review, I will not discuss all of these JA responses. Instead, I will describe how mutants of *Arabidopsis*, and other plants, have provided the tools to establish the biochemistry and cell biology of JA synthesis and its conversion to the active hormone jasmonoyl-isoleucine (JA-Ile), as well as to discover the central role of JA in coordinating plant defense against herbivores and necrotrophic pathogens. Mutants have also demonstrated the requirement for JA in stamen and pollen maturation in *Arabidopsis*, and allowed the identification of protein degradation by the ubiquitination/26S proteasome pathway as the core mechanism of JA signaling.

## 2. The genetic approach in plant biology

Analysis of mutations is a major connection path from genetics or molecular genetics to plant physiology and biochemistry. Mutated genes map genetic structure to protein function and to the physiology of the plant. In contrast to many alternative approaches, mutant analysis offers the potential to provide clear and unequivocal information about how gene products affect plant function. Once a single mutation has been established in an otherwise uniform wild-type genetic background, it follows that all the differences between the mutant and wild-type must be related directly or indirectly to the mutation. Of course, pleiotropic or compensatory effects may occur in the mutants, but once identified, these effects will also contribute to our understanding plant biology. Mutant analysis has refined our understanding about the roles of JA in plant biology, revealing surprising effects on reproductive biology (Ishiguro et al., 2001; McConn and Browse, 1996) and pathogen susceptibility (Glazebrook, 2005; Vijayan et al., 1998), helping to define the pathway and regulation of JA synthesis (Ishiguro et al., 2001; Koo et al., 2006; Stintzi and Browse, 2000) and providing the first clues about the mechanism of JA perception (Xie et al., 1998).

The screening and isolation of mutants based on a recognizable phenotypic characteristic, such as resistance to coronatine, or the sterility of *Arabidopsis* JA mutants (see below), is the traditional approach that has become known as "forward genetics". However, the abundance of DNA sequence information and the ability to infer putative gene functions based on sequence comparisons means that an investigation of biochemical and biological function can now also begin with the gene sequence – "reverse genetics". The hallmark of a reverse-genetic approach is that it begins by examining nucleotide sequences to identify an interesting genetic locus, then either locates a plant line with a mutation in the locus (T-DNA insertion mutants or TILLING, for example) or creates a plant line with altered activity of the locus by transgenic approaches (antisense, RNA interference, etc.), and then examines the plant phenotype. This information-first approach turns on its head the traditional genetic method of tracing the source of an interesting phenotype back to the mutated gene that caused it. Completion of the *Arabidopsis* genome sequence greatly enhanced these methods for isolating lines defective in expression of particular genes.

*Arabidopsis* plants containing randomly inserted T-DNA elements were originally used, in combination with mutant screens, for forward genetics (gene tagging) (Azpiroz-Leehan and Feldmann, 1997; Sanders et al., 2000; Stintzi and Browse, 2000). The use of transposons to generate insertion mutations is a conceptually similar approach. Using transposons which preferentially transpose to adjoining regions of the chromosome, a relatively small region of the *Arabidopsis* chromosome can be mutagenized to near saturation (Ito et al., 1999). However, the T-DNA lines have been particularly useful as tools for reverse genetics. The first approaches depended on PCR amplification of arrayed pools of DNA isolated from these lines using oligonucleotide primers specific for the T-DNA and the gene of interest (Azpiroz-Leehan and Feldmann, 1997), but identification of insertion mutants is particularly powerful now that the sequence of the genome has been completed. Several laboratories have systematically sequencing the *Arabidopsis* DNA flanking T-DNA insertions – recovered by thermal asymmetric interlaced PCR (TAIL-PCR) – and made these available through databases (www.arabidopsis.org) that can be queried with a gene of interest (Alonso et al., 2003). Reverse-genetics approaches are particularly useful when sequence analysis identifies a family of homologous sequences and suggests that the encoded enzymes may have overlapping (redundant) biochemical functions. Characterization of some steps in the JA-synthesis pathway depended on the identification of mutants by reverse genetics including *opcl1*, *acx1* and *acx5* (see below).

Chemical mutagenesis has been at the core of genetic analysis in *Arabidopsis* for 40 years (Rédei and Li, 1969), and the power of reverse genetics can now be brought to bear on mutations created by this classic technique. In the TILLING method (McCallum et al., 2000), a sequence of interest is amplified using different fluorescent markers to label each strand of the PCR product. When these amplified markers are hybridized to genomic DNA isolated from a population of plant lines mutagenized with ethyl methane sulfonate and then digested with a heteroduplex-specific endonuclease, probes hybridized to wild-type genomic sequences are unchanged, but probes mismatched with the induced mutations are cleaved and their altered size can be detected on agarose gels. From a heavily mutagenized population of plants it is possible to identify a group of plant lines, which are mutated in the target genes. The development of TILLING and adaptation of the technique to methods suitable for use in mass screening (Colbert et al., 2001) means that a wide range of mutant phenotypes (an allelic series) may be recovered in a single experiment, allowing a more-complete analysis of gene function.

## 3. Key mutants in JA synthesis and perception

The first mutant line deficient in JA synthesis was generated for a quite different purpose. Because chloroplasts contain very high levels of trienoic fatty acids (16:3 and 18:3), it had been inferred that they have a vital role in maintaining photosynthetic function. A triple mutant of *Arabidopsis* was produced by combining mutations at three loci: *fad3*, which encodes an endoplasmic-reticulum 18:2 desaturase, and *fad7* and *fad8*, which encode two chloroplast 16:2/18:2 desaturases (McConn and Browse, 1996). The resulting *fad3 fad7 fad8* plants contain no detectable 16:3 or 18:3 fatty acids. The triple mutant plants are indistinguishable from wild-type in vegetative growth and development at 22 °C, although photosynthesis and growth are affected under some physiological conditions (Routaboul et al., 2000; Vijayan and Browse, 2002). The *fad3 fad7 fad8* mutant does not synthesize JA because it is deficient in the precursor, 18:3 (α-linolenic acid). Characterization of this triple mutant and other JA-synthesis mutants has been central to the discovery of the many roles of JA hormone in regulating defense, development and other processes in plants (see below).

An equally important mutant to our understanding of JA biology, *coi1*, was identified in a screen for mutants of *Arabidopsis* resistant to coronatine (Feys et al., 1994). Coronatine is a bacterial phytotoxin produced by some strains of *Pseudomonas syringae*. As well as being resistant to coronatine, *coi1* mutants are insensitive to JA and male-sterile (Feys et al., 1994). Subsequent studies have confirmed that the *coi1-1* allele is very substantially deficient in genetic and biochemical responses to JA, and susceptible to attack by