An Arabidopsis Mutant Defective in Jasmonate Response Is Allelic to the Auxin-Signaling Mutant *axr1*[1]

Iskender Tiryaki[2] and Paul E. Staswick*

Department of Agronomy and Horticulture, University of Nebraska, Lincoln, Nebraska 68583–0915

A screen for Arabidopsis mutants that were insensitive to methyl jasmonate (MeJA) in an assay for seedling root growth yielded only alleles of previously isolated mutants *jar1* and *coi1*, with one exception. Mapping of the locus and morphological characterization of the new mutant suggested it might be allelic to *axr1*, which had not previously been reported to show resistance to MeJA. The F_1 from a cross of the new mutant with *axr1-3* did not show complementation, confirming that these are the same genes. The new allele is called *axr1-24*. In addition to MeJA and indole-3-acetic acid (IAA), *axr1-24* had decreased sensitivity to 1-aminocyclopropane-1-carboxylic acid, 6-benzylamino-purine, epi-brassinolide, and abscisic acid. Both *axr1-24* and the previously characterized *axr1-3* allele were shown to be susceptible to the opportunistic pathogen *Pythium irregulare*, a trait found in other jasmonate response mutants, including *jar1-1*. The double mutant *jar1-1/axr1-3* was more resistant to inhibition of root growth by MeJA and was more susceptible to *P. irregulare* infection than either single mutant, suggesting these genes might act in independent response pathways. In contrast, resistance to IAA in the double mutant was not different from *axr1-3*. Northern-blot analysis showed that IAA induced the jasmonate-responsive *lipoxygenase 2*, *AOS*, and *AtVSP* gene transcripts and induction was strongly impaired in *axr1-3*. However, transcript induction by MeJA was only minimally affected in *axr1-3*. This study demonstrates that in addition to auxin signaling, the *AXR1* locus is involved in MeJA response, providing a mechanistic link between jasmonate and auxin-signaling pathways.

Plant hormones control a diverse array of plant responses affecting growth and development, defense against microorganisms and insects, and protection from abiotic stresses (Davies, 1995). These plant signals interact with each other in both complementary and antagonistic ways to accomplish their signaling roles. Many hormone response mutants have been isolated, and characterization of some of these has revealed further evidence for interactions among plant hormones at the level of signal transduction.

Jasmonate 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), and in limiting damage from abiotic agents (Overmyer et al., 2000; Rao et al., 2000). The emerging evidence indicates that jasmonate signaling involves a complex interaction between several cyclopentanone derivatives of linolenic acid metabolism, including jasmonic acid (JA), methyl jasmonate (MeJA; Seo et al., 2001), and the JA precursor 12-oxo-phytodienoic acid (Mueller, 1997; Stintzi et al., 2001). Related products of other synthetic pathways are probably involved as well (Weber et al., 1997).

Three Arabidopsis loci have been identified in screens for resistance to MeJA or the bacterial phytotoxin coronatine, which mimics jasmonate activity. Mutations in *JAR1*, *COI1*, and *JIN1* lead to reduced sensitivity in the inhibition of root growth by MeJA and various other jasmonate-associated defects, but not to insensitivity to several other tested hormones (Staswick et al., 1992; Feys et al., 1994; Berger et al., 1996). Mutation of a mitogen-activated protein kinase (*mpk4*) also shows defects in some jasmonate responses (Peterson et al., 2000), although this may by a downstream component that is involved in response to other signals as well. Mutations in some of these genes impair only portions of the jasmonate response. For example, whereas JA is required for male fertility and *coi1-1* is male sterile (McConn and Browse, 1996; Stintzi and Browse, 2000), all known *jar1* alleles are fertile (Staswick et al., 2002). We recently demonstrated that *JAR1* does not encode a signal transduction component, but rather, an enzyme that biochemically modifies JA (Staswick et al., 2002). The inhibition of root growth by MeJA may integrate many of the subprograms that are modulated by jasmonates (Berger et al., 1996), thus additional loci affecting jasmonate response may await discovery.

Auxin is key a hormone that controls plant growth and development, and is involved in cell division and elongation. Identification of the defective genes

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[2] Present address: Department of Agronomy, Kahramanmaras Sutcu Imam University, Kahramanmaras, 46060 Turkey.

* Corresponding author; e-mail pstaswick1@unl.edu; fax 402–472–7904.

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from several auxin response mutants has led to a model for auxin response involving an ubiquitin-proteasome pathway that includes an SCF-type E3-ubiquitin ligase complex (Gray and Estelle, 2000). Interestingly, *COI1* encodes an F-box protein that is related to the TIR1 component of the auxin-signaling SCF complex. This suggests that jasmonate and auxin use a similar signaling mechanism. However, *coi1* is not altered in its response to auxin (Feys et al., 1994), suggesting that these are separate signaling pathways. This study was initiated to isolate and characterize new mutants that affect response to jasmonate. The results revealed that jasmonate and auxin act through a common signaling intermediate that also affects response to other plant hormones.

RESULTS

Isolation of a New MeJA-Insensitive Mutant

A screen of about 200,000 M_2 seedlings representing around 50,000 M_1 parents for resistance of root growth to inhibitory concentrations of MeJA yielded only alleles of the previously isolated mutant loci *jar1* and *coi1*, with one exception. The exception was crossed to wild type (*Ler*). Analysis of 270 of the resulting F_2 progeny showed a χ^2 value of 0.11 ($P = 0.73$) for a 3:1 segregation ratio (MeJA sensitive:MeJA resistant), indicating that this was a recessive single-gene mutation.

The new mutant also had a phenotype distinct from that of other jasmonate response mutants. Plants were shorter, had crinkled leaves, and exhibited partial male sterility. A detailed analysis the phenotype is presented in Table I. This phenotype contrasts with the jasmonate response mutants *jar1*, *coi1*, and *jin1* that all appear indistinguishable from wild type, except that *coi1-1* is male sterile. All 52 of the F_2 MeJA-resistant seedlings that survived transfer to soil and grew to maturity exhibited the aberrant phenotype, whereas MeJA-sensitive plants did not. This indicated that a single gene was involved in both jasmonate response and the distinct phenotype. Thus, the new mutant appeared to define a novel locus that is associated with response to MeJA.

Table I. *Morphology of wild-type and axr1-24 plants*

Parameter	Wild Type	*axr1-24*
Height (cm)	41.2 ± 3.7[a]	27.2 ± 3.9
No. of inflorescences	4.8 ± 1.0	6.1 ± 1.1
No. of branches	13.1 ± 3.9	23.1 ± 11.6
Distance between siliques (cm)	1.0 ± 0.1	0.6 ± 0.1
No. of siliques	154 ± 73	22 ± 30
Length of siliques (cm)	1.5 ± 0.1	0.6 ± 0.1
Etiolated seedling hypocotyl length (cm)	12.9 ± 1.8	12.8 ± 1.9
No. of pollen grains/flower	2,389 ± 719	680 ± 565

[a]Mean ± SD (n = 12 plants).

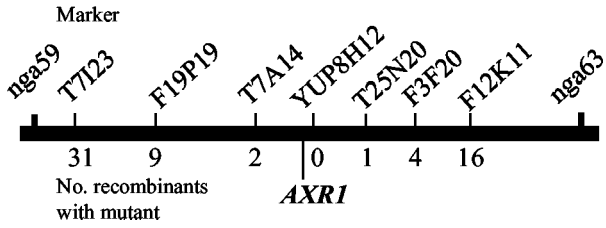

Figure 1. Mapping of the MeJA resistance locus. Molecular markers used are indicated above the chromosome I interval that is depicted. Numbers below denote the number of recombinants between the mutant locus and the respective marker. The relative position of *AXR1* is shown.

The MeJA-Insensitive Mutant Is Allelic to *axr1*

The phenotype of our new mutant was strikingly similar to that of the previously described *axr1-3* allele of the auxin response mutant (Lincoln et al., 1990). Furthermore, the locus was mapped to an interval of about 134 kb on chromosome 1 that is flanked by SSLP markers T7A14 and T25N20 (Fig. 1). *AXR1* is located within this interval, suggesting these might be the same genes. The F_1 cross of our mutant with *axr1-3* showed noncomplementation, producing only mutant plants. These results confirmed that these are the same loci and our new mutant is hereafter called *axr1-24.*

Several alleles of *axr1* have been isolated, including *axr1-3*, which displays only partial loss of gene function (Lincoln et al., 1990). We sequenced two independent cDNA clones of *axr1-24* but found no evidence of a mutation that would alter protein translation. This suggests that the mutation in *axr1-24* might be in a noncoding region that affects gene expression. The level of resistance to auxin (50% inhibition of root growth) for *axr1-24* was essentially the same as in *axr1-3* (Fig. 2A). Comparison of the phenotype of *axr1-24* (Table I) with the published results for *axr1-3* (Lincoln et al., 1990) also indicated that these alleles were similar in the severity of their defect. Together, these results suggest that the phenotype of *axr1-24* is attributable to a partial loss of gene function.

axr1-24 Is Defective in Its Response to Several Plant Hormones

Previous studies documented that *AXR1* confers sensitivity to ethylene and cytokinin as well as auxin (Timpte et al., 1995), but a role in MeJA response had not previously been reported. The MeJA dose response of *axr1-24* in primary root growth was tested over a range of concentrations and compared with wild type and *jar1-1* (Fig. 2B). *axr1-24* had a level of resistance that was less than that seen in *jar1-1* over all concentrations tested. At about 10 μM in *axr1-24*, 50% inhibition of growth occurred, whereas the concentration for 50% inhibition was 5- to 10-fold higher in *jar1-1*.

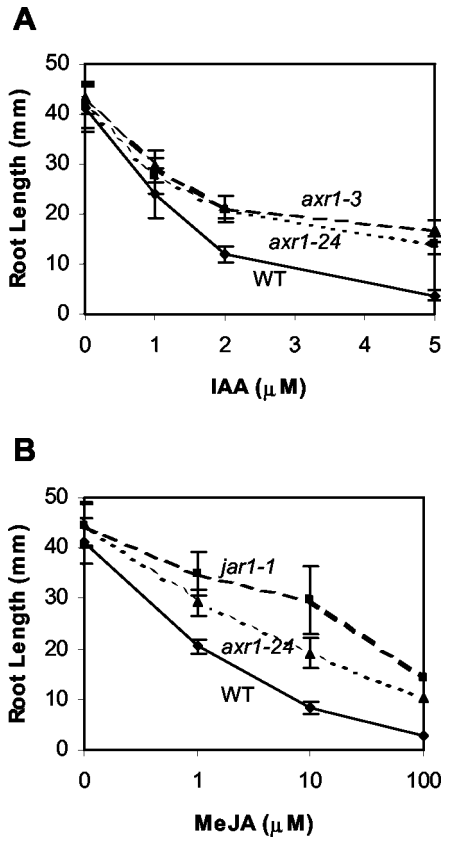

Figure 2. Dose response curve for root growth inhibition on IAA and MeJA. Wild-type (wt) and mutant (*jar1-1*, *axr1-24*, and *axr1-3*) seedling root length was measured after 10 d growth at 21 C. Error bars indicate SD ($n = 20$). A, Growth on IAA. B, Growth on MeJA.

To further investigate the hormone insensitivity of *axr1-24*, seedling root growth was tested on a range of concentrations of 6-benzylamino-purine (BA), epibrassinolide (BR), and the ethylene precursor 1-aminocyclopropane-1-carboxylic acid (ACC). The response to abscisic acid (ABA) was also examined in a seed germination assay.

ACC inhibited root growth in *axr1-24* to a level intermediate between that of wild type and the ethylene-insensitive mutant *etr1-1* (Fig. 3A). Root elongation was inhibited 8%, 50.4%, and 85.3% for *etr1*, *axr1-24*, and wild type, respectively, at 5 μM ACC. No difference among the genotypes was observed on control medium.

Brassinolides are powerful inhibitors of root growth and development. Prolonged growth in the presence of BR caused root curling, so measurements were taken after 5 d rather than 10 d of growth. *axr1-24* was resistant to both 1 and 10 nM BR, whereas strong inhibition was observed for both mutant and wild type at 100 nM (Fig. 3B).

In the assay for germination in the presence of ABA, *axr1-24* was more resistant than wild type at 0.5 μM ABA (Fig. 3C), although inhibition was similar to wild type at concentrations above 5 μM (data not shown). *axr1-24* was less resistant to ABA than the well-characterized mutant *abi1*, which germinates even at 10 μM ABA.

The effect of cytokinins on root elongation in *axr1-24* was also examined. *axr1-24* was more resistant in its response to BA than wild type. Total inhibition of root elongation was 66.4% and 73.7% for *axr1-24* and wild type, respectively, at 1.5 μM BA. Although the magnitude of difference was small compared with the other hormones tested, an analysis of variance indicated the root length was significantly different ($P < 0.05$).

axr1-24 Is Resistant to Other Inhibitors of Root Growth

The results indicated that *axr1-24* has an altered sensitivity to all tested plant hormones. To further explore the specificity of this locus in sensitivity to other chemicals, we tested root growth response in

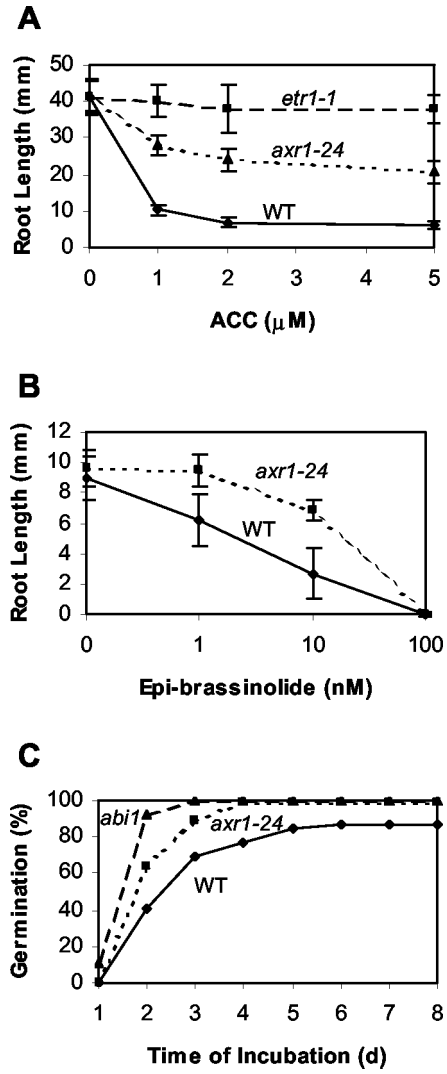

Figure 3. Insensitivity of mutants to hormones. Root length determined as in Figure 2. A, Inhibition of root growth by the ethylene precursor ACC. B, Inhibition of root growth by BR. C, Seed germination on 0.5 μM ABA.

the presence of various compounds that inhibit root growth.

axr1-24 was more resistant than wild type ($P < 0.01$) at 30 and 50 μM salicylic acid (SA), although the differences were small compared with most of the plant hormones described earlier (Table II). *axr1-24* was also resistant to the inhibitory effects of both ferulic and gallic acids. On the other hand, no difference from wild type was observed over a range of concentrations for gentisic acid, arachidonate, linolenate, juglone, and patulin (data not shown).

axr1-3 Is More Susceptible to the Fungus *Pythium irregulare*

A primary function of the jasmonates is in plant stress response, including defense against the opportunistic fungal pathogen *P. irregulare* (Staswick et al., 1998; Vijayan et al., 1998). Having determined that the locus responsible for defects in MeJA response in our mutant is in fact *AXR1*, we next tested whether the well-characterized *axr1-3* was defective in this jasmonate-requiring pathogen defense response. Inoculation of 5-week-old plants at the root zone with an isolate of *P. irregulare* caused the symptoms of wilting and tissue collapse reported previously for *jar1-1* but not seen in wild type (Staswick et al., 1998). However, symptoms appeared later in *axr1-3* than in *jar1-1* (Fig. 4), suggesting a weaker defect in this defense-signaling response compared with *jar1-1*. One-half of the *jar1* plants displayed symptoms by 8 to 9 d after inoculation, whereas *axr1-3* did not show a similar level of symptoms until 12 to 13 d. Similar results were also found for *axr1-24* (data not shown). This is consistent with the lower level of insensitivity to MeJA observed in the root growth assay for *axr1-24* compared with *jar1-1* (Fig. 1). Two-week-old plants showed symptoms earlier, but the timing of symptom appearance in *axr1-3* was still later than for *jar1* (data not shown). These results demonstrate that in addition to its involvement in MeJA-suppressed root growth, *AXR1* is necessary for resistance to *P. irregulare* in Arabidopsis.

Table II. *Resistance of axr1-24 seedling root growth to various organic acids*

Means with different letters (a and b) indicates significant difference between genotypes for each concentration ($P = 0.01$).

Acid	Concentration	Seedling Root Length	
		Wild type	*axr1-24*
	μM	mm[a]	
Salicylic	0	39.5 ± 3.4	38.7 ± 2.6
	30	14.9a ± 0.9	18.8b ± 1.1
	50	13.3a ± 1.0	16.9b ± 1.4
Ferulic	0	46.4 ± 1.7	47.6 ± 1.1
	15	24.1a ± 1.18	31.0b ± 1.4
	25	19.5a ± 0.9	27.5b ± 1.4
Gallic	0	49.7 ± 1.8	51.7 ± 1.5
	15	26.7a ± 1.5	32.0b ± 2.1
	25	22.1a ± 1.6	29.0b ± 1.3

[a]Mean ± SD ($n = 20$).

Table III. *Resistance to IAA and MeJA in jar1-1, axr1-3, and jar1-1/axr1-3*

Means with different letters (a, b, c, and d) between genotypes are significantly different ($P = 0.01$).

Genotype	Root Length		
	Control	IAA (2 μM)	MeJA (50 μM)
	mm[a]		
wt	29.1a ± 0.9	9.7a ± 0.8	6.3a ± 0.4
jar1	29.1a ± 1.1	9.4a ± 0.8	13.5c ± 1.2
axr1	29.9a ± 1.6	20.3b ± 1.5	10.1b ± 1.5
jar1/axr1	35.4b ± 1.6	19.7b ± 1.6	27.9d ± 2.1

[a]Mean ± SD ($n = 20$).

The Effect of *JAR1* and *AXR1* Is Additive

Our results showed that *AXR1* provides a link between jasmonate and auxin signaling. To investigate whether *JAR1* and *AXR1* are involved in the same or in distinct response pathways, a double mutant homozygous for both *jar1-1* and *axr1-3* was constructed. The double mutant was phenotypically similar to *axr1-3* in all aspects except flowering time, which was about 1 week earlier than in either parent (data not shown). The roots of *jar1-1/axr1-3* were about 3.4 times longer than wild type on 50 μM MeJA, and nearly the same as the length of wild type on control media (Table III). The effect of the lesions in *jar1* and *axr1* appears to be additive, because the roots of the double mutant grew to about twice the length of either single mutant on 50 μM MeJA. Although root elongation of the double mutant was also slightly greater than wild type on control medium, the difference was small compared with that seen after growth on MeJA-containing medium. Response to 2 μM indole-3-acetic acid (IAA) was the same as for *axr1-3* and *axr1-3/jar1-1* (Table III), which is consistent with the fact that *JAR1* is not known to influence auxin response.

To determine whether the pathogen response of the double mutant was also altered, its response to *P. irregulare* infection was compared with the single mutants. Figure 4 shows that symptom appearance was earlier in *jar1-1/axr1-3* than in *jar1* and *axr1-3*, although by 14 d essentially all plants were affected in both *jar1-1* and *jar1-1/axr1-3*. In contrast, about 22% of *axr1* plants appeared healthy 14 d after inoculation (Fig. 4).

Expression of Early Auxin and Jasmonate-Responsive Genes in *axr1-24*

The link between auxin and jasmonate response through *AXR1* prompted an examination of MeJA- and auxin-induced gene expression in wild type and in *axr1-24* seedlings. Jasmonate-inducible *AOS*, *li-*

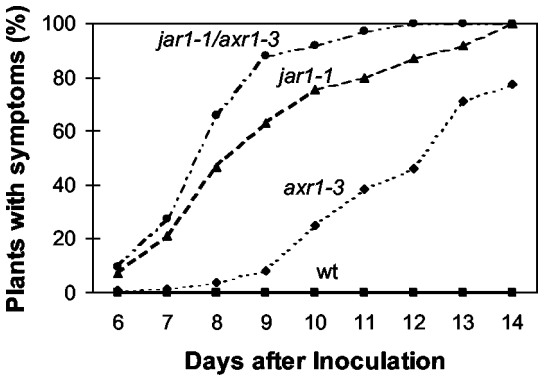

Figure 4. Infection of mutant and wild-type seedlings with *P. irregulare*. One hundred and fifty-three seedlings were used per genotype. The percentage of plants with symptoms at the times indicated is shown for each genotype. Wild type did not show symptoms during the duration of the experiment.

poxygenase 2 (*LOX2*), and *AtVSP* transcripts were elevated by MeJA treatment in wild type with a maximum level reached by about 5 h (Fig. 5A). This was followed by a decline in transcript abundance, which is consistent with the transient elevation previously reported for *AOS*, *AtVSP*, and several other jasmonate-inducible transcripts in Arabidopsis (Titarenko et al., 1997; Laudert and Weiler, 1998). These transcripts were also elevated to a similar level in *axr1-24*, although the time for maximal induction was somewhat delayed relative to wild type. MeJA treatment did not raise the mRNA level for the auxin-inducible gene *IAA1* in either genotype. Surprisingly, *LOX2*, *AOS*, and *AtVSP* were also induced by IAA, although the maximum transcript level was somewhat less than when induced by MeJA. Induction of these genes by IAA was suppressed in *axr1-24*, as was also the case for *IAA1*.

DISCUSSION

This report describes the identification and characterization of a novel Arabidopsis mutant that was defective in its response to MeJA but with additional characteristics that were atypical of those previously identified in jasmonate response mutants. The new mutant had phenotypic abnormalities in plant growth and development and was insensitive to multiple plant hormones. This contrasts with other jasmonate response mutants that, with the exception of male sterility in *coi1* (Feys et al., 1994), appear phenotypically normal and are resistant only to jasmonates. Except for male sterility, mutants defective in JA biosynthesis are also developmentally normal, indicating that JA does not play a major role in most aspects of growth and development in Arabidopsis (McConn and Browse, 1996; Sanders et al., 2000).

The discrepancy between results for other jasmonate response mutants and those reported here was resolved by the finding that the new mutant was allelic to the previously characterized auxin response mutant *axr1*. This was surprising because, although known to modestly affect ethylene and cytokinin response (Timpte et al., 1995), *axr1* was not previously documented to alter response to jasmonates. The fact that JA is essential for pollen maturation and anther dehiscence in Arabidopsis suggests that the partial male sterility observed in *axr1* alleles may reflect an impairment in development that is mediated by JA, rather than by auxin. In contrast, most other phenotypic abnormalities associated with *axr1* alleles are likely not related to jasmonate signaling.

Previous studies have identified numerous genes that act in concert with *AXR1* in a ubiquitin-like proteasome pathway that mediates auxin signaling

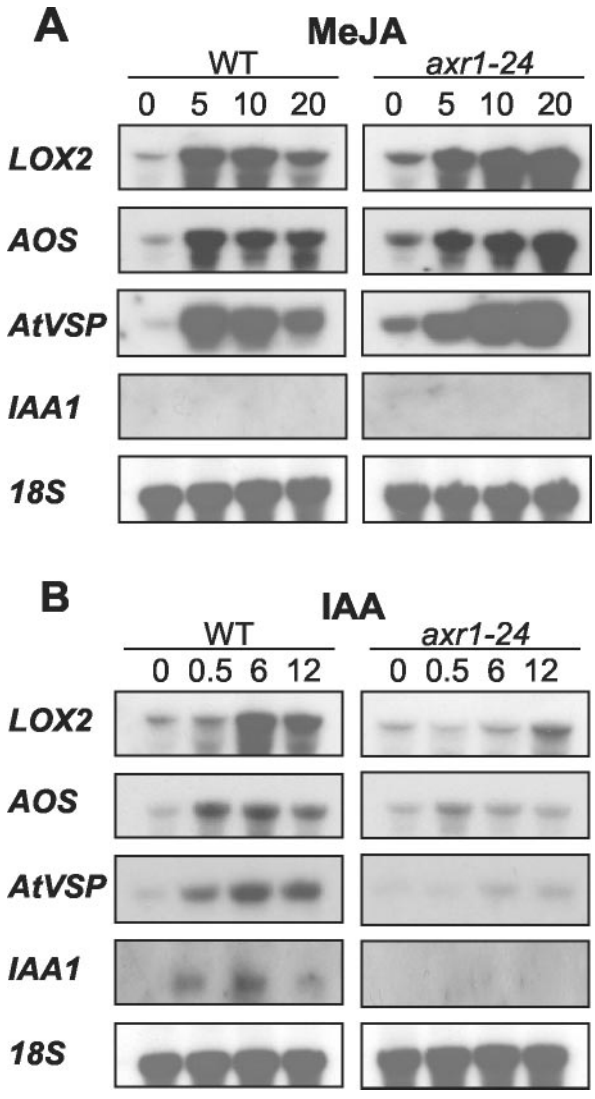

Figure 5. Transcript level for *LOX2, AOS, AtVSP,* and *IAA1* in light-grown wild-type (WT) and *axr1-24* seedlings. Numbers above lanes indicate time (h) after treatment was initiated. Hybridization probe for each row of samples is indicated to the left. A, Total RNA isolated from volatile MeJA-treated seedlings. B, RNA isolated after spraying with 1 μM IAA.

in a manner that is still not fully understood (Ruegger et al., 1998; Yeh et al., 2000). AXR1 and ECR1 form a heterodimeric enzyme that activates the ubiquitin-like RUB protein (del Pozo et al., 2002). Activated RUB is then conjugated to cullins (del Pozo et al., 1998), which are in turn components of a ubiquitin E3 ligase complex called SCF. Along with cullin, the SCF complex consists of SKP1, RBX1, and an F-box protein. SCF is involved in the transfer of ubiquitin from ubiquitin ligase to target proteins in a variety of signaling paths, the ubiquitination specificity being determined by unique SCF components. In the case of auxin signaling, the F-box protein is 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. However, *coi1* is male sterile and does not show altered sensitivity to auxin (Feys et al., 1994), whereas *tir1* is resistant to auxin and fertile (Ruegger et al., 1998), suggesting that COI1 and TIR1 function in largely independent proteasome-signaling pathways.

Our present results demonstrate that the auxin and jasmonate proteasome pathways are directly connected through AXR1, which acts upstream of TIR1/COI1. RUB modification of cullins is also important for SCF function in species other than Arabidopsis. Therefore, we hypothesize that altered jasmonate signaling in *axr1* is attributable to impaired RUB activation by AXR1/ECR1, which leads to defects in RUB modification of a cullin family member of the SCF-COI1 complex. Mutants affecting other proteins of the auxin proteasome-signaling pathway could be used to test this hypothesis, and the results would further clarify the relationship between auxin and jasmonate response.

An unresolved question is why the effect of an AXR1 mutation on jasmonate response is relatively weak compared with the auxin-related phenotype. As mentioned earlier, it is possible that partial male sterility observed in *axr1* is attributable to defects in JA signaling. However, inhibition of root growth and gene induction by MeJA was minimal compared with *jar1-1*, and pathogen susceptibility was also less than in *jar1-1*. It should be noted that insensitivity of *jar1* alleles to MeJA is itself weak compared with *coi1* (Staswick et al., 2002).

The minimal effect on JA responses may simply reflect the fact that like *axr1-3*, *axr1-24* is apparently a weak allele. The phenotype of these two alleles was similar, and we did not find evidence for a mutation in the *AXR1*-coding sequence from *axr1-24*, suggesting that (possibly minor) changes in *AXR1* gene expression are involved. Another explanation could be that there is gene redundancy for *AXR1* function in jasmonate response. Recent evidence suggests that a closely related Arabidopsis gene, called *AXL1*, may partially complement the function of *AXR1* (del Pozo et al., 2002). The extent of complementation might vary in different tissues, possibly explaining why even severe *axr1* alleles exhibit no defects in embryogenesis. AXR1 may similarly be relatively less important than AXL1 in RUB activation of cullins that are involved in SCF complexes associated with jasmonate response.

That *AXR1* is necessary for defense against the opportunistic pathogen *P. irregulare* suggests that *AXR1* plays an important role in jasmonate-mediated responses. The mechanism of resistance to this microorganism is not known, but it has been suggested that a disruption in the induction of jasmonate-regulated defense genes may lead to increased susceptibility in mutants that are impaired in jasmonate response pathways (Staswick et al., 1998; Vijayan et al., 1998). Although jasmonate-induced transcripts were elevated by MeJA in *axr1-24* (Fig. 5), subtle differences in the timing of induction might account for the low level of pathogen susceptibility we observed. We cannot rule out the possibility that auxin also plays a role in resistance to *P. irregulare*. Analysis of other auxin-signaling mutants, such as *tir1* and *ask1*, as well as auxin biosynthetic mutants should help to answer this question.

The induction of two genes involved in JA biosynthesis by IAA was unexpected. The fact that *LOX2* and *AOS* induction by IAA was suppressed in *axr1-24* may indicate another link between jasmonate and auxin signaling, in this case at the level of jasmonate synthesis. It has been proposed that there is positive feedback regulation on the expression of *LOX2* and *AOS* because JA elevates expression of both genes, and their expression is suppressed in JA response mutants (Berger et al., 1995; Mueller, 1997; Laudert and Weiler, 1998). This might provide a mechanism for the amplification of the JA signal at the level of jasmonate biosynthesis. Our results indicate that auxin might also have a role in regulating JA level.

Cross-resistance of mutants to multiple hormones is well documented (Wilson et al., 1990; Hobbie and Estelle, 1994) and suggests that the action of hormones is coordinated by common intermediates or modulators. But the physiological role of loci involved in multihormone response is less clear. Our results suggest that earlier evidence for the involvement of *AXR1* in response to other hormones may be functionally important. In addition to jasmonates, other signals critical for plant disease resistance include ethylene and SA. In some cases ethylene complements the role of JA, whereas SA usually acts in defense pathways distinct from, or even antagonistic to, those mediated by jasmonate and ethylene (Penninckx et al., 1996; Bowling et al., 1997). Although not explicitly tested here, the fact that *axr1-24* is impaired in its response to ACC and SA raises the interesting possibility that *AXR1* may help to integrate diverse defense pathways that are mediated by jasmonates, ethylene, and SA.

As for MeJA response, the effect of *axr1-24* on response to other hormones was modest compared with auxin, which is consistent with earlier results for certain other *axr1* alleles (Timpte et al., 1994; Nagpal et al., 2000; Rahman et al., 2001). The degree of insensitivity to ACC and ABA in *axr1-24* was less than that found in the respective hormone response mutants used as positive controls in this study. Furthermore, decreased sensitivity in *axr1-24* was seen even for some compounds not recognized to have specific hormone-like activity (Table II). In some of these responses, the effect of an *AXR1* mutation may be indirect, rather than the direct result of a signaling defect. Previous studies found no difference between wild type and *axr1* for GA-stimulated hypocotyl elongation (Collett et al., 2000), leading to the conclusion that auxin, ethylene, and GA independently control hypocotyl elongation. However, recent evidence suggests that the GA-response gene *SLY1* is related to F-box proteins, raising the possibility that GA signaling also involves a ubiquitin-proteasome pathway with a potential role for AXR1 or a related protein (McGinnis et al., 2002).

In summary, we have provided new evidence that jasmonate signaling involves a ubiquitin-proteasome pathway. Furthermore, our results demonstrate that this pathway is dependent on a component of the RUB-activating enzyme, AXR1, which is shared with the auxin proteasome-signaling pathway. This result provides important new insight into the mechanistic basis for interactions between auxin and jasmonate signaling in plants.

MATERIALS AND METHODS

Plant Material and Growth Conditions

Arabidopsis seeds were sown in Redi Earth (W.R. Grace, Cambridge, MA) in 8- × 8- × 8-cm plastic pots. Pots were covered with plastic film for 3 d and subsequently watered from above. Plants were grown at 21°C under continuous fluorescent illumination (approximately 100 μE m^{-2} s^{-1}). All morphological measurements were made on 9-week-old plants as described (Lincoln et al., 1990). Pollen grains were counted with a hemocytometer, and the development of stamens and pistil were examined as described previously (Estelle and Somerville, 1987).

Seedling root growth was assayed on Murashige and Skoog basal-salt agar plates as described previously (Staswick et al., 1998). All hormones and compounds were diluted in ethanol and filter sterilized, except that SA was dissolved in ddH_2O, BA in 1 N NaOH, and juglone and patulin in ethyl acetate. Each was added to sterile medium at the concentrations indicated for each experiment. The plates were chilled for 2 d at 4°C before being placed vertically in a continuously illuminated incubator at 21°C. The root length of 20 seedlings for each genotype was measured 10 d later, and each experiment was repeated at least four times. Germination on ABA was assayed on Murashige and Skoog agar medium at 21°C as described previously (Staswick et al., 1992).

Mutant Screening and Gene Mapping

Ethyl methanesulfonate-mutagenized-M_2 seeds (Columbia) were purchased from Lehle Seeds (Round Rock, TX) and screened for MeJA insensitivity as described earlier (Staswick et al., 1992). Gene mapping was done by crossing the homozygous recessive mutant to Landsberg (NW151 and CS3078), and recessive MeJA-resistant F_2 seedlings were rescued to soil. DNA from individual plants was assayed to assess the allelic status for each of several SSLP markers. An initial analysis of 133 plants placed the mutant locus between markers nga59 and nga63 on chromosome 1. Higher resolution mapping was done with 408 plants using new SSLP markers that were developed from the Cereon Genomics (Cambridge, MA) database of SNPs (http://www.Arabidopsis.org/cereon/index.html). Sequence intervals that included about 500 bp flanking the polymorphic sites were used to create primers using Seq Web, v1.2. Genomic DNA was isolated according to the methods of Yu and Pauls (1994) and was amplified using conditions previously described Bell and Ecker (1994).

Assay for Infection by *Pythium irregulare*

P. irregulare was grown and inoculated to soil containing 5-week-old wild-type and mutant plants using the same inoculation technique as described previously (Staswick et al., 1998) except that one agar plug was used for each seedling. Each pot contained nine seedlings, and 17 pots were used for each genotype. After inoculation, plants were returned to the growth chamber and monitored daily for symptoms of loss of turgor and tissue collapse.

RNA Isolation and Assay

Surface-sterilized seeds were germinated on sterilized 3MM gel-blot paper supported by a glass plate placed in a nearly vertical orientation in Magenta-boxes that contained 50 mL of liquid Murashige and Skoog basal-salt medium. Seedlings were grown 2 weeks under the same conditions as for root assays and then treated either by spraying with 1 μM IAA or with MeJA volatilized from 3MM paper taped to the inside of the container lid. Boxes were returned to the incubator and tissue samples were collected at the time intervals indicated and frozen at −80°C. Total RNA was isolated, and hybridizations were at 42°C and washes at 0.1× SSC at 62 C as described before (Staswick et al., 1998).

All probes for hybridization were generated with [^{32}P]dCTP by random primer labeling using gel-purified DNA. The *IAA1* probe was generated using PCR primers CGGAGCACAAGAAGAAC AAC (forward) and ATGGAACATCACCGACCAAC (reverse) based on the sequence of accession no. L15448. The probes for Arabidopsis allene oxide synthase (*AOS*) and vegetative storage protein (*AtVSP*) were obtained from mRNA by reverse transcriptase-PCR using primers based on the published sequences (accession no. AB007647; Staswick, 1999). The LOX2 probe was generated by PCR from the cloned Arabidopsis cDNA (Bell and Mullet, 1993). Equal loading of RNA was verified by ethidium bromide staining and by rehybridizing the blots with an 18S rDNA probe.

Generation of *jar1*/*axr1* Double Mutant

axr1 homozygotes were identified among F_2 progeny of a cross between *axr1-3* and *jar1-1* by their distinct abnormal development compared with *jar1-1* (Lincoln et al., 1990; Staswick et al., 1992). The genotype of the *JAR1* locus in putative double mutants was established with a CAPS marker for the mutant allele (P.E. Staswick, unpublished data). The double mutant was tested on both IAA- and MeJA-containing medium as described previously.

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