Two Methyl Jasmonate-Insensitive Mutants Show Altered Expression of *AtVsp* in Response to Methyl Jasmonate and Wounding[1]

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Jasmonates are plant signal molecules that are derived from lipids through the action of lipoxygenase. Jasmonates regulate gene expression during plant development and in response to water deficit, wounding, and pathogen elicitors. The signal transduction chain that mediates jasmonate action was investigated by isolating and studying two methyl jasmonate (MeJA)-insensitive mutants of *Arabidopsis thaliana*. The recessive mutants, *jin1* and *jin4*, are nonallelic and neither corresponds to *coi1*, a previously identified MeJA-insensitive mutant. Both mutants showed reduced sensitivity to MeJA-mediated root growth inhibition as well as reduced MeJA induction of *AtVsp* in leaves. Expression of *AtVsp* in flowers was not altered in the mutants. Furthermore, MeJA modulation of the jasmonate-responsive lipoxygenase and phenylalanine ammonia lyase genes was not altered in the mutants. *jin4* plants exhibited increased sensitivity to abscisic acid in seed germination assays, whereas *jin1* plants showed wild-type sensitivity. Neither mutant showed altered sensitivity to ethylene in hypocotyl growth inhibition assays. *jin1* and *jin4* identify genes that modulate the response of *AtVsp* to MeJA in leaves of *A. thaliana*.

Jasmonic acid and its derivatives, collectively referred to as jasmonates, are naturally occurring derivatives of plant lipids. These substances are synthesized from linolenic acid in a lipoxygenase-dependent biosynthetic pathway (Vick and Zimmerman, 1984). The distribution of endogenous jasmonates in plants and the actions of exogenously applied jasmonates show that these compounds regulate gene expression during plant development and in response to stress (for review, see Creelman and Mullet, 1995).

Jasmonates inhibit seed germination and growth of seedlings and roots (Yamane et al., 1981). Furthermore, tuberonic acid, a derivative of jasmonic acid, has been suggested to regulate tuber formation in potato (Koda, 1992). Jasmonates also stimulate tendril coiling in *Bryonia dioica*, a reaction that is also induced in response to touch stimulus (Falkenstein et al., 1991). Jasmonate concentrations greater than 45 μM induce senescence (Weidhase et al., 1987). In excised barley leaves, the MeJA-induced senescence response includes degradation of chlorophyll, inhibition of synthesis of photosynthesis-related proteins such as the small and large subunits of Rubisco and proteins of the light-harvesting complex, and induction of new protein synthesis (Herrmann et al., 1989; Reinbothe et al., 1993). However, the in vivo role of jasmonates in senescence is not clear because endogenous jasmonate levels are high in young, developing tissues. In soybean, the highest levels of jasmonates are found in fruits, flowers, and young leaves (Creelman and Mullet, 1995).

Jasmonate levels vary during development, in different tissues, and in response to environmental factors. Jasmonate can accumulate in plants in response to water deficit and wounding (Creelman et al., 1992; Creelman and Mullet, 1995). In plant cell cultures, jasmonate levels increase after elicitor treatment (Gundlach et al., 1992). Applied jasmonates induce a variety of genes that are also activated by wounding, drought, and pathogens. Several of the jasmonate-inducible genes encode proteins involved in plant defense (Farmer and Ryan, 1992; Reinbothe et al., 1994). Examples of jasmonate- and wound-inducible genes include those encoding chalcone synthase (Creelman et al., 1992), Phe ammonia lyase (Gundlach et al., 1992), RIP60 (Chaudhry et al., 1994), and certain proteinase inhibitors (Farmer et al., 1992). The soybean VSPs are induced by jasmonate as well as by fruit removal, wounding, and water deficit (Wittenbach, 1982, 1983; Mason and Mullet, 1990). One of the VSPs is a lipoxygenase, an enzyme involved in jasmonate biosynthesis (Tranbarger et al., 1991). The VSPs also include the acid phosphatases, VSPα and VSPβ (DeWald et al., 1992).

The signal transduction pathway that modulates jasmonate accumulation and MeJA-responsive genes requires further clarification. Promoter regions that confer jasmonate inducibility have been identified for the potato *PinII* and soybean *VspB* genes. Both of these jasmonate-responsive DNA domains contain a G box (Kim et al., 1992; Mason et al., 1993). A similar DNA element has been found in *VspA* and *Chs*, two other jasmonate-inducible genes (Schulze-Lefert et al., 1989; Mason et al., 1993).

Abbreviations: MeJA, methyl jasmonate; MS, Murashige-Skoog; VSP, vegetative storage protein.

Analysis of plant mutants is a useful approach for studying hormone action and signal transduction (Klee and Estelle, 1991). The first identified jasmonate-insensitive mutant, jar1, was isolated by screening plants for jasmonate-insensitive root growth (Staswick et al., 1992). This mutant also showed reduced accumulation of a 29-kD MeJA-inducible protein that is immunologically related to the soybean VSPα/VSPβ (Staswick et al., 1992). A second jasmonate-insensitive mutant, coi1, was isolated because the plants were resistant to coronatine, a chlorosis-inducing toxin (Feys et al., 1994). This mutant also lacked the ability to induce proteins of 29 and 31 kD when treated with MeJA. The 29- and 31-kD proteins were subsequently identified as VSPs immunologically related to the soybean VSPα and VSPβ (Benedetti and Turner, 1995; Berger et al., 1995). To further dissect the signal transduction pathway that mediates jasmonate action, we have isolated and characterized two additional MeJA-insensitive mutants of *Arabidopsis thaliana, jin1* and *jin4*.

MATERIALS AND METHODS

Plant Material and Treatments

Arabidopsis thaliana ecotype Columbia seeds were treated with 50 krad of γ-irradiation. Self-fertilization produced M_2 seeds that were used for screening. In addition, the T-DNA insertion collection (stock no. 3115, T_4 progeny) from the Arabidopsis Biological Resource Center at Ohio State University (Columbus) was screened. For screening, sterilized seeds were sown on square agar plates of MS medium (Murashige and Skoog, 1962) supplemented with 1% Suc, 0.5 g/L Mes, and 0.8% agar containing 75 μM MeJA, incubated at 4°C for 2 d, and placed vertically in a growth chamber at 21°C in continuous light. Selected seedlings were grown to maturity in soil and allowed to self-fertilize, and seeds of the next generation were rescreened for root growth on 10 and 100 μM MeJA and on MS medium in comparison to the wild type. M_4 *jin1* plants and T_6 *jin4* plants were used for crosses with wild type and with the mutants *coi1* and *jar1*. Characterization of genetic crosses was performed on medium containing 10 μM MeJA as described above.

For subsequent analyses M_4 *jin1* seeds and T_6 *jin4* seeds were used, and experiments were repeated with seeds from the next selfed generation. To examine the effect of ethylene, sterilized seeds were sown on MS medium and stored at 4°C for 4 d in the dark. The plates were then placed vertically in a 20-L sealed glass container in the dark at 22°C, and 20 μL of ethylene were added. For characterization of ABA sensitivity, sterilized seeds were planted on medium containing 5 μM ABA, incubated at 4°C for 2 d, and placed horizontally at 21°C in continuous light. Plants used to test the influence of MeJA on gene expression were grown in 20 mL of liquid MS medium in a GA7 vessel (Magenta, Chicago, IL) at 21°C in continuous light. After 15 d, MeJA was added to the liquid to a final concentration of 60 μM (stock solution 1000× in ethanol). Leaves and roots were harvested between 8 and 48 h after MeJA addition. For wounding experiments, plants were grown for 4 weeks

in soil at 21°C with a 12.5-h/11.5-h light/dark period. The upper halves of rosette leaves were wounded with a hemostat, and wounded leaves were harvested 8 h after wounding.

RNA Analysis

Tissue was frozen in liquid nitrogen immediately after harvest and stored at −80°C. Nucleic acid was isolated and analyzed as described previously (Berger et al., 1995). Detection of *AtVsp, Lox1,* and *Lox2* mRNA was performed as described previously (Bell and Mullet, 1993; Berger et al., 1995). For detection of *PalI* mRNA, the gene-specific insert from the clone *Pal 1–6* (Wanner et al., 1995) was used as a probe.

Protein Analysis

Proteins were isolated as described previously (Berger et al., 1995), separated on 15% SDS-polyacrylamide gels, and electroblotted onto an Immobilon P membrane (Millipore). Detection of antigen-antibody complexes was performed using the ECL system (Amersham) according to the manufacturer's protocol.

RESULTS

Isolation of Mutants and Genetic Analysis

A population of 40,000 Arabidopsis M_2 seeds (ecotype Columbia) mutagenized by γ-irradiation, as well as the mutant collection generated by K. Feldman (University of Arizona, Tucson) by *Agrobacterium*-mediated T-DNA insertion into Arabidopsis (ecotype WS), were screened for MeJA-insensitive root growth. Seeds were germinated on 75 μM MeJA, and after 10 to 14 d, plants with longer roots were selected. Two mutants, *jin1* and *jin4*, were further characterized. *jin1* was derived from the γ-irradiation-treated population, and *jin4* was obtained from the T-DNA insertion collection.

The effect of different MeJA concentrations on root growth in the wild types and the mutants was determined. Root growth of wild-type plants was inhibited 83 and 92% by 10 and 100 μM MeJA, respectively (Table I). Root growth of the mutants was also inhibited by MeJA but to a smaller extent. *jin1* had roots 2 times longer than wild-type plants at 10 and 100 μM MeJA. *jin4* roots were 2.5 times longer than wild-type roots at 10 μM MeJA and slightly longer

Table I. *Inhibition of root growth by MeJA*

Root length of wild-type and *jin1* and *jin4* seedlings on medium containing the MeJA concentration indicated. Seedling age was 8 d. Results are means ± SD of at least 23 seedlings. WtC, Wild-type Columbia; WtWS, wild-type WS.

MeJA	Root Length			
	WtC	jin1	WtWS	jin4
μM		*mm*		
0	45.9 ± 5.4	39.0 ± 8.4	44.4 ± 6.2	46.0 ± 3.7
10	8.0 ± 3.0	16.4 ± 5.6	9.7 ± 2.5	24.9 ± 5.1
100	3.8 ± 1.3	8.1 ± 2.3	5.2 ± 1.8	8.7 ± 2.6

than wild-type roots at 100 μM MeJA (Table I). In the absence of MeJA, the growth of each mutant was similar to the growth of the corresponding wild-type plant.

To determine whether the mutant phenotypes were inherited as recessive or dominant traits, *jin1* and *jin4* were crossed to the wild-type ecotypes Columbia and WS, respectively. The F_2 populations produced by self-fertilization of F_1 plants were scored for root growth on 10 μM MeJA. This concentration was chosen because the difference between wild-type and mutant phenotypes was maximal at this concentration (Table I). For each mutant the segregation ratio of the wild-type phenotype to the mutant phenotype was close to 3:1 (Table II), the expected ratio if the mutant phenotypes were inherited as single, recessive Mendelian markers.

To find out whether the two mutants are allelic, *jin1* and *jin4* were crossed and plants of the F_2 population were monitored for root growth on 10 μM MeJA. If the mutants are nonallelic, a ratio of 9:7 of wild-type to mutant phenotype is expected. Table II shows that the observed ratio is in agreement with *jin1* and *jin4* being nonallelic. Two other mutants with MeJA-insensitive root growth, *jar1* and *coi1*, have been described previously (Staswick et al., 1992; Feys et al., 1994). To determine whether *jin1* or *jin4* is allelic to either of these mutants, genetic crosses were done and F_2 plants from each cross were monitored for root growth on 10 μM MeJA. Of 131 plants from the F_2 population of the cross between *coi1* and *jin1*, 78 showed a jasmonate-sensitive root growth phenotype (Table II). This result indicates that *coi1* and *jin1* are nonallelic. Similarly, 90 of 139 F_2 plants from the cross between *jar1* and *jin1* had a wild-type root growth phenotype in the presence of MeJA (Table II). This ratio of wild-type to mutant phenotype is somewhat higher than expected but is consistent with the conclusion that these mutants are nonallelic (χ^2 = 4.2, P > 0.01). The higher than expected proportion of wild-type plants is probably related to our difficulty of scoring the *jar1* phenotype. Under our conditions, 42% of the homozygous *jar1* parental plants showed wild-type root lengths in the presence of MeJA.

Analysis of F_2 plants from the cross *coi1* and *jin4* revealed 44 of 117 F_2 plants (38%) with wild-type sensitivity to MeJA (Table II). This is less than the 56% wild-type plants expected for nonallelic mutations. To further examine the relationship between these mutants, F_1 plants from a cross of *coi1* and *jin4* were analyzed. In this case, all of the plants were sensitive to MeJA, indicating that *coi1* and *jin4* are nonallelic.

The most difficult cross to analyze was between *jin4* and *jar1*. This is most likely due to the weak phenotype of both mutants. Analysis of F_2 plants from this cross showed that 25% of the plants had a wild-type phenotype (Table II). If these genes were allelic, then no wild-type F_2 plants would be observed (assuming accurate scoring). Further information was obtained by examining root growth of F_1 plants from this cross (Table II). For nonallelic mutations, we would expect all of the F_1 plants to have the wild-type phenotype (Table II). However, 30% of the F_1 plants showed a MeJA-insensitive root growth phenotype (Table II). We believe that this ambiguity is due at least in part to the difficulty we encountered in correctly scoring *jar1* homozygotes as mutant, but it is not clear that this entirely accounts for the segregation anomalies seen in the *jar1* × *jin4* cross. More complex speculations are possible, but mapping of the *jin4* and *jar1* mutations will eventually resolve the question of allelism.

Sensitivity to ABA and Ethylene

Ethylene, ABA, and MeJA can inhibit plant growth. Ethylene and jasmonates promote fruit ripening and senescence. Several plant genes involved in protection from pathogens or insects respond synergistically to jasmonate and ethylene (Xu et al., 1994). ABA and jasmonate levels increase in response to water deficit and some jasmonate-inducible genes are also responsive to ABA. So far, it is unknown whether jasmonate and other plant hormones share common signal transduction pathways. Therefore we investigated whether the mutants *jin1* and *jin4* have an altered response to ethylene or ABA.

Table II. *Genetic analyses of jin1, jin4, coi1, and jar1 jasmonate-insensitive mutants*

Crosses were performed using the wild type or the first mutant written as the female parent. Plants were grown on 10 μM MeJA for 8 d (10 d for F_1 plants) and scored as sensitive or insensitive to MeJA based on root length in comparison to root length of seedlings of the homozygous parents. The expected number of each type in each cross is based on the assumption that all the mutations are recessive and nonallelic. WtC, Wild-type Columbia; WtWS, wild-type WS; –, cannot be calculated.

| Cross	Type	Total	Sensitive		Insensitive		χ^2
			Observed	Expected	Observed	Expected	
WtC × *jin1*	F_2	98	71	74	27	24	0.34
WtWS × *jin4*	F_2	172	134	129	38	43	0.78
jin1 × *jin4*	F_2	128	74	72	54	56	0.13
coi1 × *jin1*	F_2	131	78	74	53	57	0.77
jar1 × *jin1*	F_2	139	90	78	49	61	4.21
coi1 × *jin4*	F_1	57	57	57	0	0	–
coi1 × *jin4*	F_2	117	44	66	73	51	16.03
jar1 × *jin4*	F_2	133	33	75	100	58	51.2
jar1 × *jin4*[a]	F_1	33	23	33	10	0	–

[a]Some of the F_1 seeds tested derived from crosses with *jin4* as the female parent.

Inhibition of hypocotyl growth of both mutants by ethylene was similar to that seen for wild-type plants (Table III). Furthermore, addition of silver nitrate (0.1–0.02 mM), an inhibitor of ethylene action, to the root growth medium did not alter MeJA inhibition of root growth (data not shown). This indicates that the inhibition of root growth caused by MeJA is not mediated through ethylene. Germination of both mutants was inhibited by ABA. However, germination of *jin4* was more sensitive to ABA than germination of the wild type. Thus, both mutants appeared to have a normal response to exogenous ethylene and *jin4* had an increased sensitivity to exogenous ABA.

Expression of Jasmonate-Responsive Genes

In mutants with defects in a jasmonate-regulated signal transduction pathway, the regulation of jasmonate-inducible genes might be altered. Therefore, we analyzed the expression of jasmonate-responsive genes in the mutants. It has been reported that the *A. thaliana* genes *AtVsp*, *Lox1*, and *Lox2* are induced by MeJA. *AtVsp* encodes a protein homologous to the soybean *VspA* and *VspB*. *AtVsp* mRNA levels increase in leaves and roots after seedlings are treated with MeJA (Berger et al., 1995). *Lox1* and *Lox2* encode lipoxygenases. *Lox1* is induced by MeJA and mainly expressed in roots (Melan et al., 1993), and *Lox2* is mainly expressed in leaves (Bell and Mullet, 1993). Additionally, we tested whether a gene encoding a Phe ammonia lyase, *PalI*, was induced by MeJA, because this gene is jasmonate responsive in other plant species (Gundlach et al., 1992). *PalI* was at most only slightly induced by MeJA in roots (data not shown), but induction was detectable in leaves (Fig. 1B). Therefore, the expression of *PalI* and *Lox2* was studied only in leaves, the expression of *Lox1* was studied only in roots, and *AtVsp* expression was analyzed in roots and in leaves.

As shown in the northern blot analyses in Figure 1, *AtVsp* mRNA levels were low in roots of all control plants. *AtVsp* was induced similarly by MeJA in roots of *jin1* and *jin4* in comparison to the wild type. The expression pattern of *Lox1* in roots of control and MeJA-treated plants of each wild type was unaltered in the corresponding mutant. In leaves, *Lox2* mRNA levels increased similarly in response
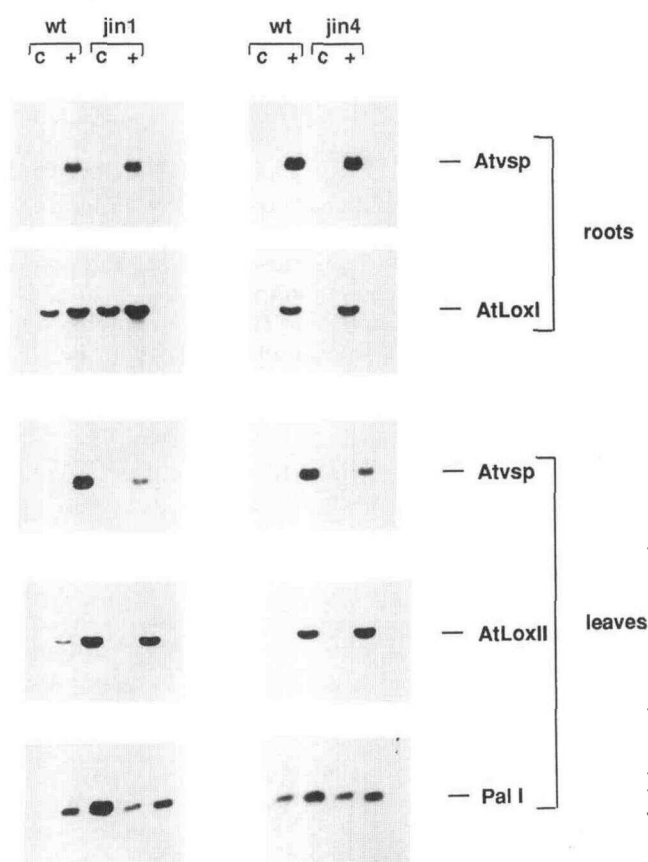
Figure 1. Accumulation of *AtVsp*, *Lox1*, *Lox2*, and *PalI* mRNA in leaves and roots of wild-type Columbia (wt), *jin1*, wild-type WS (wt), and *jin4* in response to MeJA. Plants were untreated (c) or treated with MeJA (final concentration 60 μM) for 8 h (+). Seven micrograms of total nucleic acid were loaded per lane.

to MeJA treatment in the wild type and the mutant (Fig. 1). Expression of *PalI* was increased by MeJA to a similar extent in leaves of *jin1* and *jin4* in comparison to the corresponding wild type. Strong induction of *AtVsp* mRNA was detectable after addition of MeJA to wild-type seedlings. However, expression of *AtVsp* in leaves of *jin1* and *jin4* was only slightly induced by MeJA. To determine whether this weaker induction of *AtVsp* is based on a slower response to MeJA in the mutants, *AtVsp* expression was analyzed at 12, 24, and 48 h after MeJA treatment. Figure 2A shows that *AtVsp* mRNA levels did not increase further in leaves of either mutant after 12, 24, and 48 h. In this experiment, *jin4* showed almost no induction of *AtVsp* by MeJA.

To determine whether the difference in *AtVsp* expression is also detectable at the protein level, western blot analyses with an antibody directed against soybean VSP were performed. This antibody cross-reacts with two Arabidopsis proteins of 29 and 30 kD. These proteins show an expression pattern similar to that of the *AtVsp* mRNA in that they are abundant in flowers and inducible in roots and leaves by MeJA (Berger et al., 1995). In leaves of all control plants only low levels of the immunoreactive proteins could be

Table III. *Sensitivity of jin4 and jin1 plants to ethylene and ABA*

Hypocotyl length was measured for seedlings grown for 4 d in the dark in an atmosphere of 1 μL/L ethylene. Results are percentages of hypocotyl length of control plants grown in air and are the average of two independent experiments. Germination on plates containing 5 μM ABA was scored after 12 d of growth in the light. At this time, germination of all seed types on control plates without ABA was 100%. Results are the average of three independent experiments. Thirty-six seeds were measured per experiment and seed type. WtC, Wild-type Columbia, WtWS, wild-type WS.

Value Measured	WtC	jin1	WtWS	jin4
Hypocotyl growth in 1 μL/L ethylene as a percentage of length in air	48 ± 3	40 ± 1	44 ± 5	37 ± 5
Percentage of germination on 5 μM ABA	80 ± 12	76 ± 3	72 ± 4	13 ± 5
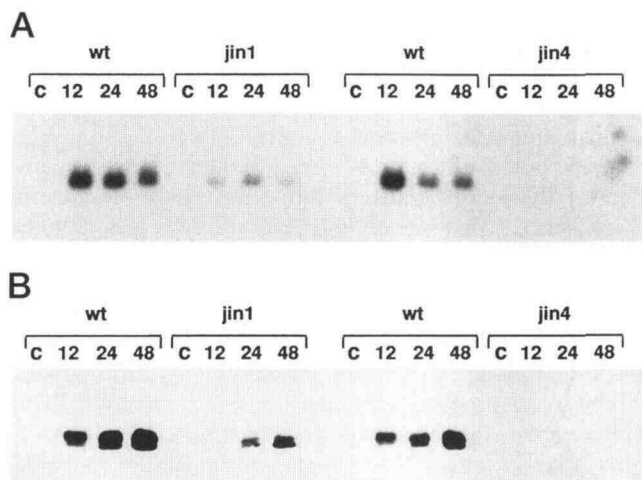
Figure 2. Accumulation of *AtVsp* mRNA (A) and VSP-related proteins (B) in response to MeJA in leaves of wild-type Columbia (wt), *jin1*, wild-type WS (wt), and *jin4*. Leaves of nontreated control plants (c) and of plants treated with 60 μM MeJA were harvested at the times indicated. A, Seven micrograms of total nucleic acid were loaded per lane. B, Proteins were separated by SDS-gel electrophoresis (12 μg of protein per lane), transferred to Immobilon P membranes, and incubated with antibody against soybean VSP.

detected (Fig. 2B). Twelve hours after addition of MeJA the 29- and 30-kD proteins increased in both wild types, and after 24 and 48 h both proteins were strongly induced. In contrast, in *jin4*, an increase of these proteins was barely detectable. In *jin1* both proteins were induced but to a smaller extent than in the wild type. It is interesting that in *jin1* the 29-kD protein was more induced than the 30-kD protein, whereas in the wild types at 12 h the 30-kD protein was more abundant and after 24 h both proteins showed the same abundance.

Wound Response

As discussed earlier, jasmonates may be involved in wound signaling. Therefore, the wound response might be altered in MeJA-insensitive mutants. To address this question, we studied the expression of two wound-inducible genes, *Lox2* and *AtVsp*. In each mutant the expression pattern of *Lox2* in control and wounded plants was similar to that seen in the corresponding wild type (Fig. 3A). In *jin1* the pattern of *AtVsp* expression was similar to the wild type. However, a weaker induction of *AtVsp* by wounding was detected in leaves of *jin4* than in leaves of wild-type plants.

To determine whether the less substantial induction of *AtVsp* expression in *jin4* by wounding is also detectable on the protein level, protein extracts of leaves from unwounded and wounded plants were tested for abundance of VSP-related proteins. The results of the western blot analysis shown in Figure 3B indicate that the 29- and 30-kD proteins were strongly induced in leaves of wild-type plants 8 h after wounding. In *jin4* only a very small increase of both proteins could be detected after wounding. In *jin1*,

both proteins clearly accumulated in response to wounding, but the induction was less than in wild-type plants.

Expression of *AtVsp* in Flowers

AtVsp is expressed in flowers at high levels (Berger et al., 1995). Since MeJA- and wound-induced *AtVsp* expression in leaves is altered in the mutants, we examined whether the constitutive expression of *AtVsp* in flowers is also changed. The northern blot in Figure 4 shows that both mutants expressed *AtVsp* in flowers at levels similar to those in wild-type plants.

DISCUSSION

In this report we have described the characterization of two recessive, nonallelic, MeJA-insensitive mutants, *jin1* and *jin4*. Root growth in both mutants is less sensitive to MeJA than in wild type. In addition, induction of the jasmonate-responsive gene, *AtVsp*, in leaves of *jin1* and *jin4* is reduced in seedlings treated with MeJA. These same responses have been previously documented for two other *A. thaliana* mutants, *coi1* and *jar1* (Staswick et al., 1992; Feys et al., 1994; Benedetti and Turner, 1995). Crosses of *jin1* and *jin4* to *coi1* demonstrated that *jin1*, *jin4*, and *coi1* affect different genes that influence plant sensitivity to jasmonate. Crosses between *jin1* and *jar1* also showed that these mutants are nonallelic. The relationship of *jin4* and *jar1* was more difficult to establish. Among all of the mutants, only *jin4* and *jar1* show increased sensitivity to ABA. A cross of *jin4* and *jar1* produced F$_1$ plants of which 30% showed a mutant phenotype in the root growth assay. If these mutants are nonallelic, then no mutant F$_1$ plants would be expected unless the products from one copy of
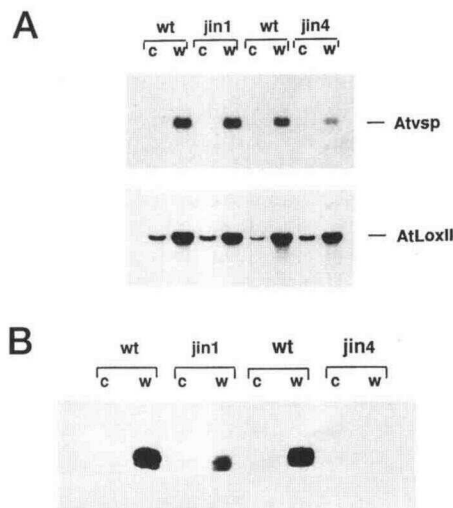
Figure 3. Accumulation of *AtVsp* and *Lox2* mRNA (A) and VSP-related proteins (B) in response to wounding in leaves of wild-type Columbia (wt), *jin1*, wild-type WS (wt), and *jin4*. Plants were untreated (c) or wounded (w) and leaves were harvested after 8 h of incubation in the light. A, Five micrograms of total nucleic acid were loaded per lane. B, Proteins were separated by SDS-gel electrophoresis (7 μg of protein per lane), transferred to Immobilon P membranes, and incubated with antibody against soybean VSP.
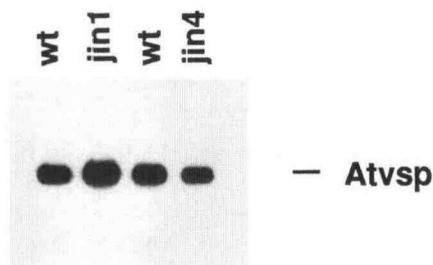
Figure 4. Accumulation of *AtVsp* mRNA in flowers of wild-type Columbia (wt), *jin1*, wild-type WS (wt), and *jin4*. Four micrograms of nucleic acid were loaded per lane.

the two mutated genes interact to create a mutant phenotype. If the genes altered in *jin4* and *jar1* are alleles, then all of the F_1 plants should have been mutant. Furthermore, if the mutated genes in *jin4* and *jar1* are allelic, then all of the F_2 plants from this cross should have been mutant. However, only 70% of the F_2 progeny from the *jin4* and *jar1* cross were scored as mutant. We believe that one problem in resolving the relationship between *jin4* and *jar1* is the difficulty of scoring the jasmonate-insensitive growth in the *jar1* plants. Under our conditions, 42% of the homozygous *jar1* parental plants were scored as wild type. Further experiments including mapping of *jin4* and *jar1* will be needed to resolve this issue.

All of the jasmonate-insensitive mutants characterized to date show reduced sensitivity to root growth inhibition by MeJA. In contrast, each mutant alters subportions of the MeJA response in leaves and flowers. For example, the *coi1* mutant is male sterile and does not induce *Vsp* in flowers, whereas *jin1* and *jin4* are fertile and *Vsp* expression is normal in flowers. This suggests that the MeJA root growth inhibition assay integrates many of subprograms that are modulated by MeJA. Unfortunately, the molecular basis of jasmonate-mediated inhibition of root growth is not known. Jasmonate levels in roots are low relative to other parts of plants (Creelman et al., 1995). Similarly, jasmonate-responsive genes such as *VspA* are expressed at very low levels in roots unless plants are treated with MeJA (Mason and Mullet, 1990). Therefore, MeJA may inhibit root growth by activating genes not normally expressed in this organ. In addition, MeJA has been reported to increase ethylene biosynthesis by inducing ethylene-forming enzyme activity in tomato fruit (Czapski and Saniewski, 1992). If this occurs in roots, then increased ethylene could inhibit root growth (Goeschl and Kays, 1975). However, we did not detect ethylene accumulation in Magenta boxes or flasks containing MeJA-treated seedlings (data not shown), and the inhibitor of ethylene action, silver nitrate, did not reverse MeJA-mediated root growth inhibition. Moreover, experiments were carried out demonstrating that the mutants retain wild-type sensitivity to ethylene, as measured by a hypocotyl growth inhibition assay.

jin1 and *jin4* show a pleiotropic phenotype that includes reduced root growth inhibition by MeJA and reduced *AtVsp* induction in leaves in response to MeJA and wounding. In both mutants, reduced induction of *AtVsp* expres-

sion was detected only in leaves; induction in roots was not altered. Furthermore, developmentally regulated expression of *AtVsp* in flowers was not changed in *jin1* or *jin4*. This argues that *jin1* and *jin4* are not mutants in *AtVsp* but instead affect factors that regulate *AtVsp* expression in response to MeJA and wounding in leaves. The affected part of the signal transduction pathway is specific for *AtVsp*, since expression of *PalI*, *Lox1*, and *Lox2* is not altered in the mutants. MeJA-induced accumulation of *Vsp* mRNA in soybean is inhibited if plants are pretreated with cycloheximide to block cytoplasmic translation (D. DeWald and J.E. Mullet, unpublished data). Similarly, MeJA-induced accumulation of *Pin2* is also blocked by pretreatment with inhibitors of proteins synthesis (Pena-Cortes et al., 1995). This suggests that MeJA initially activates transcription or translation of a gene that is required to activate transcription of the *Vsp* and perhaps other genes. The genes identified by *jin1* and *jin4* could act to modulate the lower part of this cascade, which is consistent with their somewhat selective influence on *AtVsp* expression in leaves.

The phenotypes of *jin1* and *jin4* make it unlikely that these mutants identify jasmonate receptors. In contrast, the jasmonate-insensitive mutant *coi1* exhibits jasmonate-insensitive root growth, loss of MeJA-induced *AtVsp* expression in leaves and flowers, and loss of MeJA-induced expression of *Lox* in leaves (Benedetti and Turner, 1995). These characteristics are consistent with *coi1* being upstream of *jin1* and *jin4* in the MeJA signal transduction pathway, assuming that all three genes are on the same pathway, and suggest that *coi1* may alter an MeJA receptor.

In potato and tomato, ABA has been shown to potentiate jasmonate biosynthesis in response to wounding (Pena-Cortes, et al., 1995). It is interesting that *jin4* and *jar1* plants (Staswick et al., 1992) exhibit increased sensitivity to ABA in germination assays. Therefore, although ABA potentiates jasmonate biosynthesis, jasmonate in wild-type plants may diminish the ability of ABA to repress germination. These mutants identify one component in the jasmonate signal transduction pathway that interacts with or influences the ABA signal transduction pathway.

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