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Methyl jasmonate inhibition of root growth and induction of a leaf protein are decreased in an *Arabidopsis thaliana* mutant

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ABSTRACT **Jasmonic acid and its methyl ester, methyl jasmonate (MeJA), are plant signaling molecules that affect plant growth and gene expression. Primary root growth of wild-type *Arabidopsis thaliana* seedlings was inhibited 50% when seedlings were grown on agar medium containing 0.1 μM MeJA. An ethyl methanesulfonate mutant (*jar1*) with decreased sensitivity to MeJA inhibition of root elongation was isolated and characterized. Genetic data indicated the trait was recessive and controlled by a single Mendelian factor. MeJA-induced polypeptides were detected in *Arabidopsis* leaves by antiserum to a MeJA-inducible vegetative storage protein from soybean. The induction of these proteins by MeJA in the mutant was at least 4-fold less in *jar1* compared to wild type. In contrast, seeds of *jar1* plants were more sensitive than wild type to inhibition of germination by abscisic acid. These results suggest that the defect in *jar1* affects a general jasmonate response pathway, which may regulate multiple genes in different plant organs.**

Jasmonate is an endogenous plant compound that affects growth and was recently recognized for its ability to induce the expression of specific plant genes. Methyl jasmonate (MeJA), the methyl ester of jasmonic acid (JA), is one of the few plant compounds that is effective as a vapor at low concentrations, inducing tomato leaf proteinase inhibitors (1) and soybean leaf vegetative storage proteins (VSPs) (2). These two protein classes are also wound-inducible and recent results indicate that MeJA induces the phytoalexin plant defense pathway in several species (3). This evidence suggests that jasmonate may be an important stress-signaling molecule in plants.

Jasmonate is derived from the lipoxygenase-dependent oxidation of linolenic acid (4). By analogy with the production of various eicosanoids involved in animal stress signaling (e.g., prostaglandins), it has been suggested that JA arises from the release of cell membrane fatty acids through the action of lipase in response to wounding or autolytic events (5, 6). MeJA also has pheromone activity in at least one insect (7) and is found in certain fungi. Thus, jasmonate is of general biological interest.

Evidence that *de novo* synthesis of jasmonate plays a role in regulating soybean VSP genes in response to wounding was recently reported (8). However, little is known about the signal-transduction mechanism for jasmonate action in plants. Mutants in phytohormone synthesis and response have received increased attention in recent years. Such mutants not only provide a better understanding of plant growth regulator function but, with emerging technology, may provide a strategy for the isolation of genes involved in plant hormone signaling pathways (9, 10). The purpose of this study was to find and characterize *Arabidopsis* mutants with an altered response to MeJA.

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METHODS

Mutant Screening. *Arabidopsis thaliana*, ecotype Columbia, was used in these experiments. Sterile plants were grown on an agar medium in plates as described by Lincoln *et al.* (11). Seeds were sterilized by soaking in 70% ethanol for 2 min in a 1.5-ml microcentrifuge tube, treating with 30% bleach (HOCl) in 0.05% Triton X-100 for 15 min, and then washing five times with sterile distilled water. About 500 seeds per plate were planted in three slits cut into a 0.7% agar medium containing mineral nutrient solution (11). MeJA was diluted in 100% methanol and added to the autoclaved medium. Plates were wrapped with porous surgical tape and incubated vertically in a continuously illuminated incubator at 21°C. Roots emerging from the germinated seeds penetrated the agar and did not grow on the surface of the agar.

Seeds were mutagenized by soaking about 25,000 seeds (≈50 seeds per mg) for 16 hr in 100 ml of 0.3% (vol/vol) ethyl methanesulfonate, then washed periodically for 4 hr with several volumes of water (11). M_1 seeds were sown in Redi earth mixture (W.R. Grace, Cambridge, MA) in plastic trays. Plants were grown at 18°C under continuous fluorescent illumination [≈100 $\mu E \cdot m^{-2} \cdot sec^{-1}$; 1 E (einstein) = 1 mol of photons]. Seeds were suspended in a slurry of 0.1% agar and planted in flats at about 1 seed per cm^2. Flats were covered with plastic domes for 3 days and then uncovered and subsequently watered from above.

Crosses were performed using the Columbia ecotype *glab1* mutant as the female parent to serve as a marker for inadvertent self-fertilization. To perform the crosses, sepals and petals on flower buds were peeled back and the exposed flowers were hand-emasculated. Flowers were fertilized with pollen from mutant plants.

Test of Germination. Seed was stored at least 3 months after harvest before planting. Surface-sterilized seeds were dispersed on agar plates and incubated at 4°C for 2 days. This treatment was to assure that germination was not affected by seed dormancy. Plates were then transferred to an incubator at 22°C under fluorescent light (≈100 $\mu E \cdot m^{-2} \cdot s^{-1}$; 14-hr day/10-hr night cycle). Abscisic acid (ABA) was dissolved in 50% (vol/vol) dimethyl sulfoxide and added to sterilized medium after cooling. MeJA was added from a filter-sterilized concentrated stock dissolved in water. The progression of germination was evaluated at various intervals after the beginning of incubation at the higher temperature. Plates were examined under a dissecting microscope, and a seed was considered to have germinated when the emerging primary root reached a length equal to that of the seed.

Accumulation of VSP-Like Protein. Five-week-old plants were sprayed daily with 50 μM MeJA and leaves were harvested after 3 days. At this time flower stalks had emerged but were not yet elongated. Protein extraction, quantification, and analysis by immunoblotting were as described (12).

Abbreviations: ABA, abscisic acid; JA, jasmonic acid; MeJA, methyl jasmonate; VSP, vegetative storage protein.

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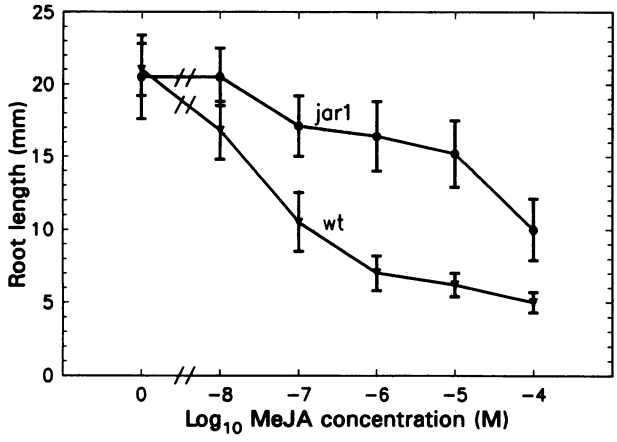

FIG. 1. Growth inhibition of primary roots by MeJA. Root length of *jar1* and wild-type (wt) plants was measured 9 days after planting. Bars indicate SE, $n = 25$.

RESULTS

Effects of MeJA on Primary Root Growth. It has been reported that MeJA inhibits root growth in some plant species (13). We examined whether primary root growth in *Arabidopsis* was sensitive to MeJA by germinating seeds embedded in agar containing various concentrations of MeJA. Nine days after planting, 50% inhibition of primary root growth occurred at about 0.1 μM MeJA for wild-type *Arabidopsis* (Fig. 1).

Because primary root growth can be easily scored in large seedling populations, we screened M_2 populations of ethyl methanesulfonate-mutagenized seeds for mutants in which primary root growth was resistant to MeJA. Among 35,000 plantlets screened on 100 μM MeJA, 4 resistant plantlets were selected. Fig. 2 shows the phenotype of one of these (*jar1*) 6 days after planting on 10 μM MeJA. Its characterization is described in this paper.

Examination of the M_3 generation of *jar1* plants revealed that the mutant was highly resistant to MeJA. Fifty percent inhibition of root growth occurred at about 100 μM MeJA (Fig. 1). In the absence of MeJA, elongation of roots for *jar1* was comparable to wild type.

Segregation analysis of progeny from the cross of wild type (*glabl*) × *jar1* demonstrated that the resistance trait was inherited as a single recessive Mendelian marker. The F_1 progeny were all sensitive to 1 μM MeJA and the segregation pattern of F_2 progeny corresponded closely to an expected 3:1 ratio (Table 1).

Induction of Specific Proteins by MeJA Is Reduced in *jar1* Plants. MeJA induces the accumulation of two soybean VSP

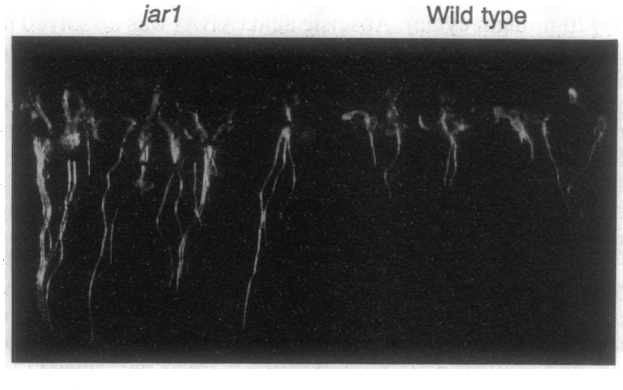

FIG. 2. Root growth of MeJA-resistant mutant (*jar1/jar1*) and wild-type (col-0) *Arabidopsis* seedlings in agar medium containing 10 μM MeJA. Photograph was taken 6 days after sowing. Mutant plants are from a single backcross population.

Table 1. Progeny analysis of the cross wild type (*glabl*) × *jar1*

	No. of seedlings		
	Total	MeJA sensitive	MeJA resistant
F_1	32	32	0
F_2	458	335	123*

*χ^2 for correspondence to a 3:1 segregation ratio of sensitive to resistant = 0.84 ($P = 0.3$–0.5).

polypeptides that function in temporary nutrient storage in leaves and other organs (14). Soybean VSP antiserum cross-reacted with two polypeptides of similar size in several plant species, including *Arabidopsis*, when plants were grown with high levels of nitrogen (P.E.S., unpublished results). We therefore examined whether the crossreacting proteins in *Arabidopsis* were inducible by MeJA as they are in soybean and whether the induction was affected in *jar1*. When plants were sprayed with 50 μM MeJA, two proteins accumulated that were similar in size to the soybean VSPs (Fig. 3). The proteins appeared to be much less abundant than in soybean. The amount of protein loaded in the lane for soybean was only 8% of that for *Arabidopsis*, yet a much stronger signal resulted in the former. Loading more total protein resulted in nonspecific staining of numerous *Arabidopsis* polypeptides. In the mutant the response to MeJA was barely detectable. This experiment was repeated five times, each yielding the same result. Comparison with a dilution standard indicated that the induced proteins were at least 4-fold more prevalent in the wild type than in the mutant. The large subunit of ribulose-1,5-bisphosphate carboxylase, the most abundant protein in the extract, also reacted weakly with the antiserum

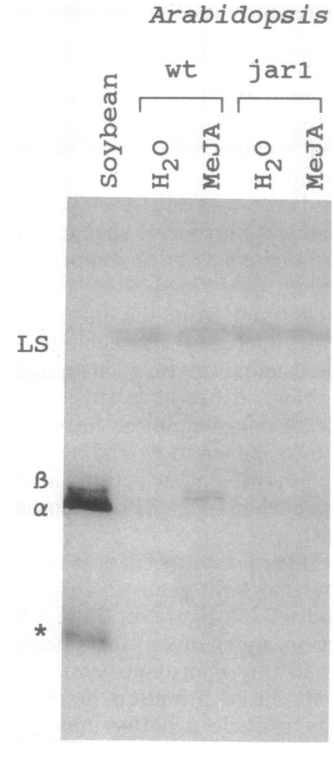

FIG. 3. Western blot analysis of specific proteins induced by MeJA in *Arabidopsis* leaves. Sixty micrograms of total leaf protein was loaded for each lane except for the soybean sample, which contained 5 μg. The latter was from a mature leaf explant incubated 3 days with 50 μM MeJA. The positions of the α and β polypeptides (27 and 29 kDa, respectively) of soybean VSP and the ribulose biscarboxylase large subunit (LS) (55 kDa) are indicated at left. The asterisk denotes a protein (≈15 kDa) thought to be a degradation product of soybean VSP. wt, Wild type.

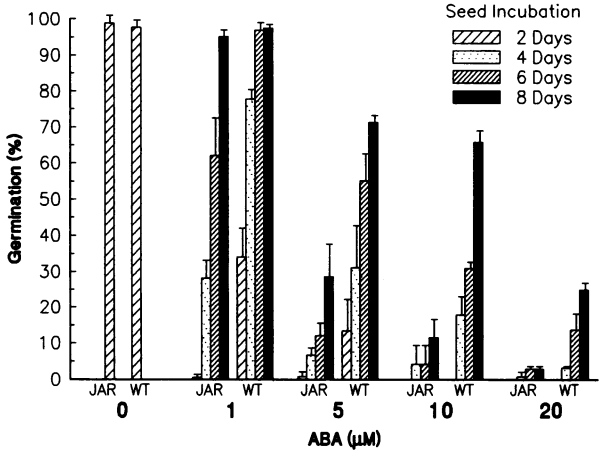

FIG. 4. Germination of *Arabidopsis* seeds in the presence of ABA. Twenty to 50 wild-type (WT) seeds were distributed on half of an agar Petri dish containing the indicated concentration of ABA. The other half of each plate contained a similar number of *jar1* seeds. Percent of seeds germinated on each plate was determined 2, 4, 6, and 8 days after incubation at 22°C. Vertical lines above each bar indicate SE for three plates.

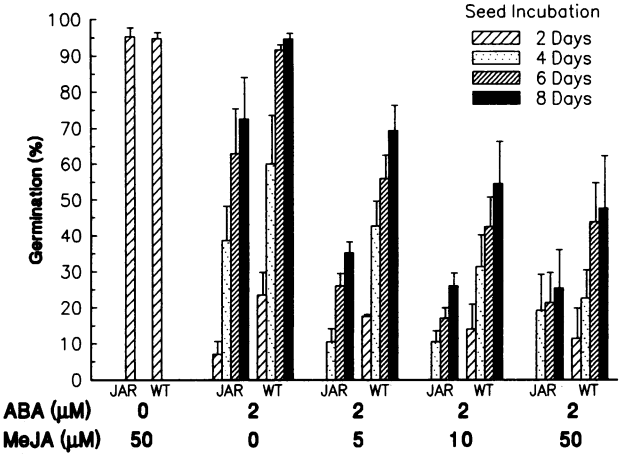

FIG. 5. Germination of *Arabidopsis* seeds in the presence of ABA and MeJA. Procedures were as described for Fig. 4.

but did not interfere with the assay. The abundance of this protein was not affected by the treatments.

***jar1* Seeds Are More Sensitive to Inhibition of Germination by ABA.** Both ABA and jasmonate have similar effects on some plant responses, including the induction of proteinase inhibitors (1, 15), inhibition of plant growth (16), and inhibition of seed germination (17). This suggests that ABA and jasmonate might utilize a common signal-transduction pathway. Since ABA inhibits germination of *Arabidopsis* seeds, and mutants that are insensitive to this effect of ABA have been isolated (18), we tested whether germination of *jar1* seeds was resistant to ABA. On the contrary, germination of *jar1* seeds was more sensitive to ABA. Fig. 4 summarizes the results of germination on agar media that contained different levels of ABA. This experiment was repeated twice with similar results. The germination rate of wild-type seeds on 20 μM ABA was about the same as that of *jar1* seeds germinated on only 5 μM ABA. In the absence of ABA there was no difference in the germination rate for *jar1* and wild type. The greater sensitivity to ABA indicates that the *jar1* mutant is not an ABA-insensitive mutant.

We further examined the relationship between *jar1* and three ABA-insensitive mutants (*abi1*, *abi2*, and *abi3*) by assaying each for the effect of 20 μM MeJA on seedling root length 6 days after germination. Table 2 summarizes the results and shows that root length of the ABA mutants was similar to wild type, and only half that of *jar1*. Thus, the ABA-insensitive mutants have little if any resistance to MeJA.

MeJA and ABA Act Synergistically to Inhibit Germination. The decreased germination of *jar1* in response to ABA suggested that ABA and MeJA might interact and affect germination. To test this, we germinated seeds in the presence of MeJA alone or with ABA and MeJA combined (Fig. 5). Germination rates in wild type and *jar1* were not affected by MeJA alone at concentrations up to 50 μM, the maximum tested. In contrast, as little as 5 μM MeJA inhibited germination when 2 μM ABA was also present. The absolute level of germination was lower for *jar1* than for wild type in the presence of ABA, but MeJA further decreased germination by about the same magnitude in both the mutant and the wild type.

Table 2. Primary root growth of ABA-insensitive mutants on MeJA

Genotype	Root length, cm
Wild type	3.75 ± 1.03
jar1	9.88 ± 1.90
abi1	4.16 ± 0.75
abi2	4.60 ± 1.03
abi3	4.27 ± 1.00

n = 6–11 plants for each genotype.

DISCUSSION

Jasmonate was first isolated from *Jasminum grandiflora* as the fragrant methyl ester, MeJA. Jasmonate and several related compounds have been studied for their inhibitory effects on growth and a variety of other activities in plants (ref. 5 and references therein). MeJA is also an active component of the female-attracting pheromone released by male Oriental fruit moths (7) and is found in certain fungi.

Jasmonate has received renewed attention because of its ability to increase the expression of certain plant genes, including some that are known to be wound inducible (1, 14). Although it has not been conclusively demonstrated that endogenous JA or its derivatives control plant gene expression, recent evidence supports this idea (8). We reasoned that if JA is a plant growth regulator it might be possible to isolate jasmonate response mutants whose characterization could help elucidate the role of JA in plants. To our knowledge this is the first report to describe such a mutant.

Mutants were selected based on the insensitivity of primary root growth to MeJA. One previous study reported that MeJA concentrations above 10 μM inhibited root growth in some species (13). Although we screened *Arabidopsis* mutants at 100 μM MeJA to identify plants highly resistant to the effects of MeJA, 50% inhibition of wild type occurred at about 0.1 μM. Inhibition of growth may be a direct effect of MeJA or a general response to external stress. The response to low levels of MeJA observed in this study supports the idea that it is a specific signaling effect, rather than a toxic response. By way of comparison, the concentration of atmospheric MeJA that induces soybean VSPs and tomato protease inhibitors is estimated to be about 0.08 μM (1).

The *jar1* mutation is pleiotropic, affecting the induction of proteins in leaves as well as root growth. Specific polypeptides that are antigenically related to soybean VSPs accumulate in wild-type *Arabidopsis* in response to MeJA. The response was at least 4-fold lower in the mutant. Soybean VSPα and VSPβ are temporary storage molecules that accumulate abundantly in vegetative organs when nitrogen is available (14, 19). The two *Arabidopsis* polypeptides that respond to MeJA are also increased in leaves of plants supplied with high levels of ammonium nitrate (P.E.S.,

unpublished results), which is the case for soybean VSP as well (8).

The abundance of the *Arabidopsis* leaf polypeptides that react with soybean VSP antiserum is apparently quite low compared with soybean leaf VSP. Sequence divergence may also contribute to the weak antigenic signal detected here. The apparent low abundance suggests that they may not play an important storage role in *Arabidopsis* as they do in soybean. Recently, it was reported that tomato contains low amounts of an acid phosphatase that has about 45% sequence identity with soybean VSP (20). The function of the *Arabidopsis* polypeptides that react with the VSP antiserum remains to be determined.

Our finding that *jar1* is less, rather than more, resistant to ABA suggests that *jar1* is not simply resistant to general stress responses. Some hormone response mutants are resistant to high concentrations of a variety of other growth regulators (21–23). It has been hypothesized that in some cases resistance to seedling growth inhibition may result from an increased rate of germination, allowing seedlings to outgrow the effects of low levels of growth inhibitor (24). This is clearly not the explanation for *jar1* insensitivity to MeJA, since insensitivity was also evident in mature leaves for the induction of specific polypeptides. Furthermore, rather than being resistant to other hormones, *jar1* is more sensitive to ABA than is wild type. The latter result suggests that ABA and MeJA may interact in some signaling pathways.

Up to 50 μM MeJA alone had no effect on seed germination in *Arabidopsis*. This contrasts with a previous report indicating that as little as 1 μM MeJA inhibited germination of both *Brassica napus* and *Linum usitatissimum* (17). The reason for the difference between species is not clear. However, we observed a synergism when ABA and MeJA were combined. This supports the idea that ABA and MeJA may interact in some biological responses. Tomato leaf proteinase inhibitors are induced by ABA (15) as well as MeJA (1) but ABA has no apparent effect on soybean VSP, either with or without JA (25).

The response of *jar1* to ABA is opposite that of the *Arabidopsis abi1*, *abi2*, and *abi3* mutants, which are insensitive to ABA (26). We also examined root growth in these mutants in the presence of 20 μM MeJA. Roots of the ABA-insensitive mutants were inhibited to about the same extent as wild type, whereas *jar1* roots were twice as long after growth on MeJA. Because of the sensitivity of the ABA mutants to MeJA, we believe the mutation in *jar1* involves a different locus than any of those responsible for ABA insensitivity. Additional work on *jar1* and other jasmonate response mutants should help clarify the mechanism(s) by which MeJA affects various responses in plants, and may lead to the isolation of the genes involved.

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1. Farmer, E. E. & Ryan, C. A. (1990) *Proc. Natl. Acad. Sci. USA* **87**, 7713–7716.
2. Franceschi, V. R. & Grimes, H. D. (1991) *Proc. Natl. Acad. Sci. USA* **88**, 6745–6749.
3. Gundlach, H., Müller, M. J., Kutchan, T. M. & Zenk, M. H. (1992) *Proc. Natl. Acad. Sci. USA* **89**, 2389–2393.
4. Vick, B. A. & Zimmerman, D. C. (1984) *Plant Physiol.* **75**, 458–461.
5. Anderson, J. M. (1989) in *Second Messengers in Plant Growth and Development*, eds. Boss, W. F. & Morré, D. J. (Liss, New York), pp. 181–212.
6. Farmer, E. E. & Ryan, C. A. (1992) *Plant Cell* **4**, 129–134.
7. Baker, T. C., Nishida, R. & Roelofs, W. L. (1991) *Science* **214**, 1359–1361.
8. Staswick, P. E., Huang, J. & Rhee, Y. (1991) *Plant Physiol.* **96**, 130–136.
9. Reid, J. B. (1990) *J. Plant Growth Regul.* **9**, 97–111.
10. Klee, H. & Estelle, M. (1991) *Annu. Rev. Plant Physiol. Plant Mol. Biol.* **42**, 529–551.
11. Lincoln, C., Britton, J. H. & Estelle, M. (1990) *Plant Cell* **2**, 1071–1080.
12. Staswick, P. E. (1989) *Plant Physiol.* **89**, 309–315.
13. Corbineau, F., Rudnicki, R. M. & Come, D. (1988) *Plant Growth Regul.* **7**, 157–169.
14. Staswick, P. E. (1990) *Plant Cell* **2**, 1–6.
15. Pena-Cortes, H., Willmitzer, L. & Sanches-Serrano, J. J. (1991) *Plant Cell* **3**, 963–972.
16. Dathe, W., Ronsch, H., Preis, A., Schade, W., Sembdner, G. & Schreiber, K. (1981) *Planta* **153**, 530–535.
17. Wilen, R. W., van Rooijen, G. J. H., Pearce, D. W., Pharis, R. P., Holbrook, L. A. & Moloney, M. M. (1991) *Plant Physiol.* **95**, 399–405.
18. Koorneef, M., Reuling, G. & Karssen, C. M. (1984) *Physiol. Plant.* **61**, 377–383.
19. Wittenbach, V. A. (1983) *Plant Physiol.* **73**, 125–129.
20. Williamson, V. M. & Colwell, G. (1991) *Plant Physiol.* **97**, 139–146.
21. Bitoun, R., Rousselin, R. & Caboche, M. (1990) *Mol. Gen. Genet.* **220**, 234–239.
22. Pickett, F. B., Wilson, A. K. & Estelle, M. (1990) *Plant Physiol.* **94**, 1462–1466.
23. Wilson, A. K., Pickett, F. B., Turner, J. C. & Estelle, M. (1990) *Mol. Gen. Genet.* **222**, 377–383.
24. Blonstein, A. D., Parry, A. D., Horgan, R. & King, P. J. (1991) *Planta* **183**, 244–250.
25. Anderson, J. M. (1991) *J. Plant Growth Regul.* **10**, 5–10.
26. Finkelstein, R. R. & Somerville, C. R. (1990) *Plant Physiol.* **94**, 1172–1179.