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Genetic Architecture of Plastic Methyl Jasmonate Responses in *Arabidopsis thaliana*

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ABSTRACT

The ability of a single genotype to generate different phenotypes in disparate environments is termed phenotypic plasticity, which reflects the interaction of genotype and environment on developmental processes. However, there is controversy over the definition of plasticity genes. The gene regulation model states that plasticity loci influence trait changes between environments without altering the means within a given environment. Alternatively, the allelic sensitivity model argues that plasticity evolves due to selection of phenotypic values expressed within particular environments; hence plasticity must be controlled by loci expressed within these environments. To identify genetic loci controlling phenotypic plasticity and address this controversy, we analyzed the plasticity of glucosinolate accumulation under methyl jasmonate (MeJa) treatment in *Arabidopsis thaliana*. We found genetic variation influencing multiple MeJa signal transduction pathways. Analysis of MeJa responses in the Landsberg *erecta* × Columbia recombinant inbred lines identified a number of quantitative trait loci (QTL) that regulate plastic MeJa responses. All significant plasticity QTL also impacted the mean trait value in at least one of the two "control" or "MeJa" environments, supporting the allelic sensitivity model. Additionally, we present an analysis of MeJa and salicylic acid cross-talk in glucosinolate regulation and describe the implications for glucosinolate physiology and functional understanding of Arabidopsis MeJa signal transduction.

An organism's genotype, its environment, and the interaction between genotype and environment determine the phenotype displayed. Phenotypic plasticity is the ability of a single genotype to produce multiple phenotypes in response to different environments and understanding the molecular basis of phenotypic plasticity is important for ecology, evolution, and plant breeding (Via *et al.* 1995; Sultan 2000). Further, genes different from those that regulate a trait's mean value may influence genotype × environment interactions in determining a phenotype. Thereby, identifying genes regulating genotype × environment interactions is necessary for understanding the molecular basis of phenotypic plasticity. One method to search for such loci is to use inbred lines to measure a quantitative trait in multiple environments and to map quantitative trait loci (QTL) that influence differential trait expression (Jansen *et al.* 1995; Crossa *et al.* 1999). Another method is to study how laboratory-induced mutations alter phenotypic plasticity (Pigliucci and Schmitt 1999). While these approaches have greatly aided our genetic understanding of phenotypic plasticity, little is known about the actual genes that naturally control genotype × environment interactions.

One reason for this molecular nescience is the complexity of the systems used to identify most genotype × environment interactions. Studies typically investigate complex physiological traits such as yield, flowering time, or seed germination and their response to complex environmental changes such as temperature or day length (Clarke *et al.* 1995; van der Schar *et al.* 1997; Alonso-Blanco *et al.* 1998; Stratton 1998). Thus, it is difficult to characterize how a gene underlying a QTL × environment interaction is involved in perceiving environmental change and consequently influencing phenotypic expression.

To identify genes controlling phenotypic plasticity, we studied the genetics of how glucosinolate secondary metabolite accumulation responds to variable environments in *Arabidopsis thaliana* (Kliebenstein *et al.* 2001c). Glucosinolates are secondary metabolites produced from tryptophan and methionine, which differentially influence Arabidopsis defense against fungal and insect attack (Halkier and Du 1997; Tierens *et al.* 2001; Kliebenstein *et al.* 2002). Further, all Arabidopsis glucosinolates are quantifiable with a single high-throughput HPLC assay and some are known to be induced by jasmonate and other hormones (Figure 1; Brader *et al.* 2001; Kliebenstein *et al.* 2001c). Thus, we can study glucosinolate plasticity in response to specific hormones as a substitute for more complex environmental changes. Finally, the genetics and biochemistry of glucosinolate biosynthesis is well understood, with a number of the enzymes cloned and characterized (Wittstock and Halkier 2000; Bak *et al.* 2001; Hansen *et al.* 2001; Klie-

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benstein *et al.* 2001a,b; Kroymann *et al.* 2001; Reintanz *et al.* 2001). The combination of candidate gene knowledge for glucosinolate biosynthesis and hormone signal transduction with complete genome sequence makes the Arabidopsis/glucosinolate system useful for identifying and cloning phenotypic plasticity loci (Farmer *et al.* 1998; Dangl and Jones 2001).

Insect feeding damage to plant tissues triggers induced responses to a variety of stimuli, including touch, wounding, regurgitant ("spit factors"), and microbial pathogens (Alborn *et al.* 2000; Reymond *et al.* 2000; Stotz *et al.* 2000; Turlings *et al.* 2000; Hermsmeier *et al.* 2001; Moran and Thompson 2001). Furthermore, insect behavior causes variable rates of damage during a 24-hr period; hence responses to herbivory may be confounded with endogenous plant circadian rhythms (Kreuger and Potter 2001). Consequently, we performed experimental studies of glucosinolate responses to plant hormones to study a simplified component of genetic variation in plastic responses. These simplified treatments and responses are not a surrogate for the full complexity of responses to natural enemies. Rather, functional and genetic analysis of glucosinolate induction is a first step toward understanding patterns and mechanisms of genetic variation in developmental plasticity.

To investigate genotype × environment interactions, we assessed how methyl jasmonate (MeJa) and salicylic acid (SA) treatments alter glucosinolate accumulation. We then characterized variation among different ecotypes/accessions for these responses and analyzed Landsberg *erecta* (L*er*) × Columbia (Col-0) recombinant inbred (RI) lines for QTL with differential impacts on glucosinolate accumulation between control and MeJa treatments (Lister and Dean 1993). Finally, we documented how these QTL alter glucosinolate regulation by MeJa.

MATERIALS AND METHODS

Plant growth and seed source: All seed stocks were obtained from the Arabidopsis Biological Resource Center (http://aims.cps.msu.edu/aims/). Two to three seeds of the appropriate line were planted in 1 cell of a 96-cell flat containing potting soil mix with timed-release fertilizer (Osmocote). After planting, flats were cold stratified at 4° for 5 days and then moved to the growth room. Three days after germination, the plants were thinned to a density of 1 plant per cell (507 plants m^{-2}) and grown under 14-hr day length with cool white and GrowLux fluorescent bulbs in a controlled environment growth room.

MeJa and SA treatment and HPLC analysis of glucosinolates: After 4 weeks of growth, plants were treated with ethanol control, SA, MeJa, or both SA and MeJa. MeJa and ethanol control treatments were done by mixing 50 g of unscented lanolin paste with 1 ml of ethanol with or without 0.5% MeJa. This mixture was then spread on the inside of a humidity top and placed on top of the 96-cell flat to be treated. SA treatment was accomplished by spraying the plants with a 400-μm solution of SA in water. As a control, the SA treatments were accompanied by an ethanol/lanolin paste treatment. The humidity top was sealed to the 96-cell flat with parafilm and the flat returned to the normal growth room. The treatments for the time course experiment were allowed to proceed for 24 and 48 hr. For the accession and RI analyses the treatments were done for 48 hr. At the end of the treatment 10 leaf discs were harvested and used for glucosinolate extraction and analysis as described (Kliebenstein *et al.* 2001a). Specific glucosinolates were identified by comparison of retention times and UV absorption spectra with purified standards. All glucosinolate absorption data (measured at 229 nm) were converted to micromoles per 100 g of fresh weight using response factors determined from the purified standards for each of the glucosinolates (Kliebenstein *et al.* 2001a).

MeJa and SA interaction experimental design: L*er* plants were singly planted in 96 6-cm pots. After 4 weeks, the 90 healthiest plants were selected and treated with SA, MeJa, MeJa and SA, water, or harvested as the 0 hr control, with 18 plants per treatment. After 24 hr one-half of the plants from each treatment were harvested for glucosinolates and the other half returned to the growth room. The remaining plants were harvested after 48 hr. This experiment was repeated three independent times with similar results in all three experiments.

Accession experimental design: The following accessions were used (abbreviation, accession, country, stock center number): Aa-0, Rhon, Germany, N900; Col-0, Columbia, N1092; Cvi, Cape Verde Islands, N902; Hodja, Khurmatov, Tadjikistan, N922; L*er*-0, Landsberg, Germany, NW20; No-0, Nossen, Germany, N1394; Pi-0, Pitztal, Austria, N1456; Sorbo, Khurmatov, Tadjikistan, N931. L*er*, Cvi, and Col-0 were selected as representatives of standard Arabidopsis accessions. The other five accessions were preselected as non-MeJa responsive from a GC-MS screen analyzing glucosinolate breakdown product formation before and after wounding and insect herbivory. These five accessions showed minimal induction of indole-3-acetonitrile after both wounding and insect herbivory (D. J. Kliebenstein and T. Mitchell-Olds, unpublished data).

A total of 12 plants from each accession were planted in a 96-cell flat in a randomized design. Two flats were planted for each experiment and kept side by side for the entire duration of the experiment. After 4 weeks, one flat was treated with MeJa and the other with the ethanol control. After 48 hr, 10 leaf discs were harvested for glucosinolate extraction and analysis. This experiment was replicated four times such that plants from any two experiments were never grown simultaneously. A total of 722 plants were analyzed in this experiment for an average of 45 plants per accession per treatment.

RI line experimental design: Lines of 94 L*er* × Col-0 RI plus L*er* and Col-0 parental controls were randomly planted in four 96-cell flats per experiment (Lister and Dean 1993). After 4 weeks, two flats were treated with MeJa and the other two with ethanol control. After 48 hr, 10 leaf discs were harvested for glucosinolate extraction and analysis. This experiment was replicated four times such that plants from any two experiments were never grown simultaneously. Due to germination problems, 7 of the 94 RI lines were removed from the analysis due to a lack of data. A total of 1034 plants were analyzed for this experiment for an average of 6 plants per line per treatment.

Statistical analysis: SAS/Stat version 8e was utilized for all statistics (SAS Institute, Cary, NC). ANOVA analysis with the general linear module was used to analyze accession by treatment effects. This was done by running the following model:

$$\text{Glucosinolate} = \text{Constant} + \text{Treatment} + \text{Replicate} + \text{Accession} + \text{Treatment} \times \text{Replicate} + \text{Treatment} \times \text{Accession} + \text{Replicate} \times \text{Accession}.$$

We also examined a complete model including all two-

way interactions and the three-way Treatment × Replicate × Accession interaction. The three-way interaction was never significant and was removed from all analysis. Least-squares means were also obtained.

Fixed effect ANOVA with the GLM module was also used to obtain least-squares means for the RI lines under the two treatments. The following model was used:

$$\text{Glucosinolate} = \text{Constant} + \text{RI line} + \text{Treatment} + \text{Replicate} + \text{RI line} \times \text{Replicate} + \text{RI line} \times \text{Treatment} + \text{Treatment} \times \text{Replicate}.$$

The RI line × Treatment least-squares means were used for QTL mapping.

To test for marker × treatment interactions, the marker that maximized the LOD score for each individual QTL was utilized in ANOVA with the previously obtained least-squares means for each RI line under MeJa and control treatments, using:

$$\text{Glucosinolate} = \text{Constant} + \text{Treatment} + \text{Marker A} + \text{Marker B} + \text{Treatment} \times \text{Marker A} + \text{Treatment} \times \text{Marker B} + \text{Marker A} \times \text{Marker B}.$$

This tests for epistatic interactions and QTL × treatment interactions. This model was expanded or simplified depending upon the number of QTL being tested. The complete data set used both the MeJa and control data for each RI line. Thus, the *P* values presented represent the likelihood that the marker controls the mean value of the trait.

QTL mapping: Composite interval mapping in QTL cartographer was utilized for all QTL mapping (Zeng 1993, 1994; Basten *et al.* 1999). Genome-wide 0.05 significance thresholds for each trait were estimated by doing 500 permutations (Churchill and Doerge 1994). Each glucosinolate was mapped as three separate traits: the level of the glucosinolate in the control treatment, the level of the glucosinolate under MeJa, and the level obtained by subtracting the control level from the MeJa level. QTL identified under all three conditions were then used for the above ANOVA analysis.

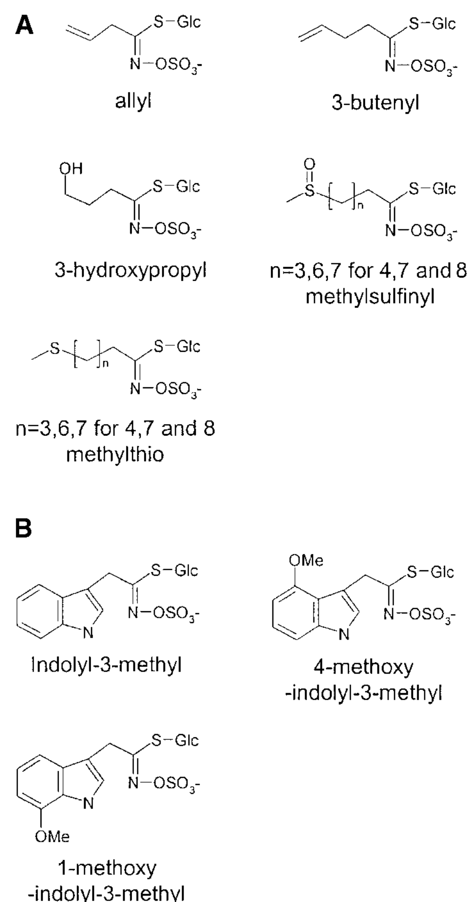

Figure 1.—Chemical structure of glucosinolates analyzed in this article. The semisystematic names are given below the structures. (A) Methionine-derived glucosinolates. (B) Tryptophan-derived glucosinolate.

RESULTS

Methyl jasmonate and salicylic acid alter glucosinolate accumulation: Leaves of the *A. thaliana* accession L*er* contain seven major glucosinolates, four derived from methionine and three produced from tryptophan, all detectable with a single HPLC assay (Kliebenstein *et al.* 2001b,c). This enables the simultaneous assessment of treatment effects on all seven glucosinolates. Treatment of L*er* with MeJa and SA identified four different glucosinolate response patterns.

The first pattern is marked by MeJa induction and includes the 3-hydroxypropyl, indol-3-ylmethyl, and 1-methoxyindol-3-ylmethyl glucosinolates (Figures 1 and 2, A and B). The MeJa induction maximized within 24 hr, after which 3-hydroxypropyl glucosinolate concentration returned to control levels while indol-3-ylmethyl glucosinolate levels remained elevated (Figures 1 and 2, A and B). These glucosinolates are not induced by SA treatment at any time (Figures 1 and 2, A and B). Further, a cotreatment of SA and MeJa completely blocked the 24-hr induction generated by MeJa alone, and this blockage was alleviated after 48 hr (Figures 1 and 2, A and B).

4-Methoxyindol-3-ylmethyl glucosinolate is the lone glucosinolate in the second response pattern, which is induced by SA and not by MeJa (Figures 1 and 2C) The SA induction nearly maximized within the first 24 hr and cotreatment of SA and MeJa blocked the SA-mediated induction at 24 hr; this impasse was mitigated after 48 hr (Figures 1 and 2C).

The third response pattern is characterized by a lack of regulation by either MeJa or SA alone, but by a significant induction at 48 hr through the combination of both hormones (Figures 1 and 2D). The sole representative of this pattern is 8-methylsulfinyloctyl glucosinolate (Figures 1 and 2D). The final response pattern includes those glucosinolates that show no regulation by any of the treatments tested, 8-methylthiooctyl and 7-methylthioseptyl glucosinolates (Figure 1).

Plasticity in MeJa-mediated glucosinolate regulation: To test whether MeJa regulation of L*er* glucosinolates extrapolates to other accessions, we assayed glucosinolate responses to MeJa in L*er* and seven additional accessions (see materials and methods for experimen-

tal design). The complete experiment was replicated four independent times and the results from all replicates were combined to test different sources of variation. ANOVA showed significant accession differences for accumulation of 11 of the 13 glucosinolate variables measured (Table 1; Kliebenstein *et al.* 2001c). The levels of 12 glucosinolate traits also showed significant variation among the four experimental replicates (Table 1). Finally, 3-hydroxypropyl-, 4-methylthiobutyl-, and indole-derived glucosinolates showed significant MeJa-mediated responses across the entire population (Table 1).

In addition to separately analyzing the impact of replicates, accessions, and MeJa, we tested their interaction effects. Significant accession by replicate and treatment by replicate interactions were found for some glucosinolates (Table 1). This heterogeneity shows the importance of environmental variation on glucosinolate metabolism.

There were also differences among accessions in the ability of MeJa to alter glucosinolate accumulation (Table 1). MeJa reduces 8-methylsulfinyloctyl glucosinolate levels in Col-0 (Figure 3A). Further, when the ratio of 8-methylsulfinyloctyl glucosinolate to the total 8C glucosinolate pool is analyzed, this MeJa-mediated decrease is highly significant (Figure 3B). In contrast, the opposite response is significant in L*er*: MeJa causes induction of 8-methylsulfinyloctyl glucosinolate levels (Figure 3B). Most accessions did not significantly alter either the level of 8-methylsulfinyloctyl glucosinolate or the ratio of 8-methylsulfinyloctyl to the total 8C glucosinolate pool (Figure 3, A and B). 4-Methoxyindol-3-ylmethyl glucosinolate levels are also reduced by MeJa treatment in Col-0 and Pi-0 (Figure 3C).

The pattern of phenotypic plasticity in total indole glucosinolate levels differs from that of 8-methylsulfinyloctyl glucosinolate (Figure 3, B and D). In this case, L*er* has the largest MeJa-mediated induction followed by Cvi and Col-0 (Figure 3D). The difference between the L*er* and Col-0 inductions is significant by the Bonferroni-adjusted two-tailed *t*-test (Figure 3D). The other accessions showed no MeJa-mediated induction of total indole glucosinolate concentration. Finally, there were no significant accession × treatment × replicate interactions.

QTL × treatment effects regulating glucosinolate accumulation: The differences in how L*er* and Col-0 regulate glucosinolate accumulation in response to MeJa suggest that several genetic loci control these differences (Figure 3). To identify these genes, we used the L*er* × Col-0 RI lines to compare QTL that regulate

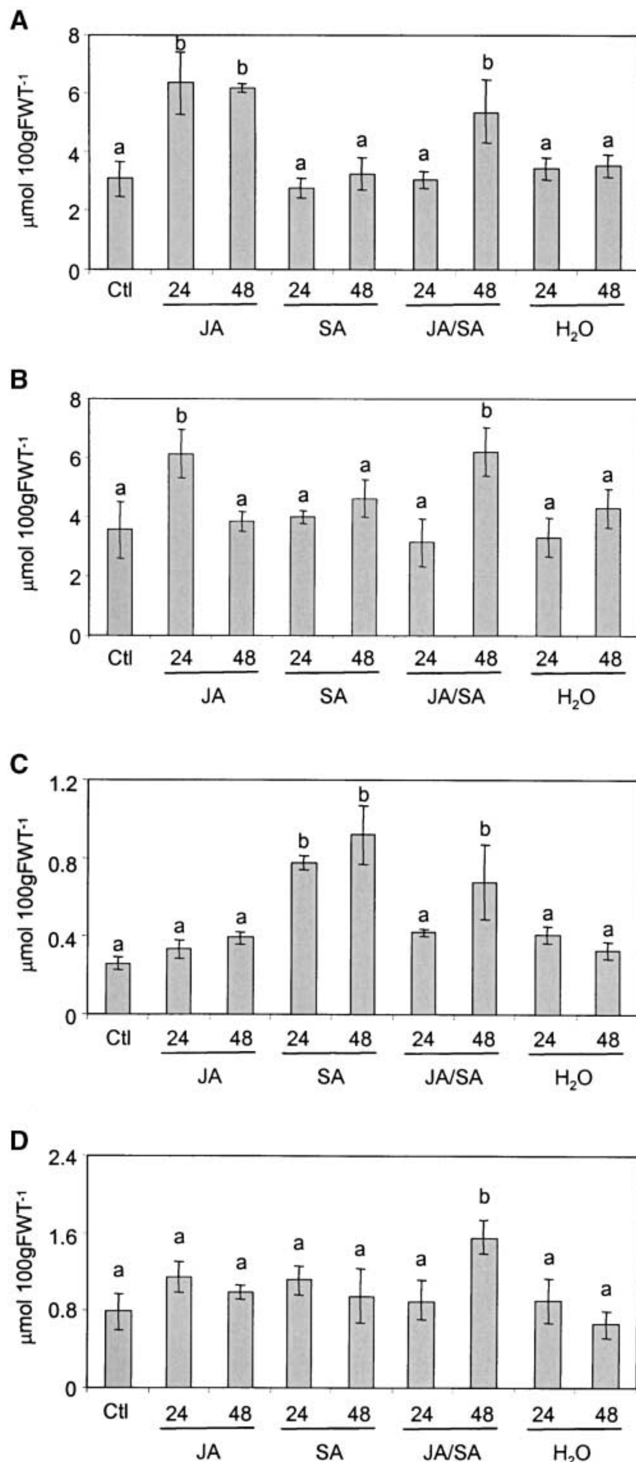

Figure 2.—Altered accumulation of L*er* glucosinolates in response to treatment with MeJa and SA. Ctl, plants taken at the start of the experiment; H_2O, control plants sprayed with water and covered with humidity tray containing lanolin paste mixed with 1 ml ethanol; SA, plants treated with SA; JA, plants treated with MeJa; JA/SA, plants treated with both MeJa and SA. The least-squares means obtained from three independent replicates are shown. Samples with different letters above the bar are statistically different at the $P = 0.01$ level. (A) Total indolic glucosinolate concentration. Indol-3-ylmethyl and 1-methoxyindol-3-ylmethyl glucosinolates display a similar pattern. (B) 3-Hydroxypropyl glucosinolate concentration. (C) 4-Methoxyindol-3-ylmethyl glucosinolate concentration. (D) 8-Methylsulfinyloctyl glucosinolate concentration.

TABLE 1

ANOVA of glucosinolate responses to MeJa treatment in eight accessions

Trait	*n*	Ecotype		Replicate		Treatment		Ecotype × replicate		Ecotype × treatment		Replicate × treatment	
		F	*P*	F	*P*	F	*P*	F	*P*	F	*P*	F	*P*
8MSO to total 8C	8	56.26	*0.0001*	118.79	*0.0001*	3.53	0.0606	2.73	*0.0001*	6.22	*0.0001*	18.52	*0.0001*
8-Methylsulfinyloctyl	8	59.92	*0.0001*	1.34	0.2601	1.64	0.2009	4.49	*0.0001*	4.69	*0.0001*	7.91	*0.0001*
4-Methoxy-indolyl-3-methyl	8	78.12	*0.0001*	65.41	*0.0001*	19.73	*0.0001*	4.31	*0.0001*	3.60	*0.0008*	32.36	*0.0001*
Total indolic	8	32.99	*0.0001*	81.25	*0.0001*	64.12	*0.0001*	3.15	*0.0001*	2.75	*0.0081*	17.69	*0.0001*
Indolyl-3-methyl	8	34.99	*0.0001*	69.26	*0.0001*	84.09	*0.0001*	3.12	*0.0001*	2.68	*0.0097*	26.80	*0.0001*
1-Methoxy-indolyl-3-methyl	8	0.94	0.4789	11.31	*0.0001*	0.48	0.4866	1.48	0.0802	2.43	*0.0190*	1.42	0.2439
Butenyl	2	2.02	0.1567	9.49	*0.0001*	0.79	0.3760	9.66	*0.0001*	5.29	*0.0226*	1.64	0.1819
8-Methylthiooctyl	8	100.76	*0.0001*	60.93	*0.0001*	0.07	0.7982	3.76	*0.0001*	1.94	0.0615	2.45	0.0623
3-Hydroxypropyl	3	8.47	*0.0003*	11.34	*0.0001*	10.52	*0.0013*	0.63	0.7039	1.47	0.2319	0.24	0.8710
4-Methylsulfinylbutyl	2	78.17	*0.0001*	12.25	*0.0001*	1.95	0.1642	1.51	0.2137	0.60	0.4393	2.45	0.0651
4-Methylthiobutyl	2	19.60	*0.0001*	51.06	*0.0001*	12.82	*0.0004*	8.79	*0.0001*	0.46	0.5001	2.97	*0.0334*
Allyl	3	82.54	*0.0001*	6.62	*0.0003*	0.39	0.5305	6.66	*0.0001*	0.58	0.5627	2.25	0.0826
Total aliphatic	8	186.42	*0.0001*	13.22	*0.0001*	2.46	0.1175	5.62	*0.0001*	0.71	0.6664	1.89	0.1294

The glucosinolate traits individually tested by ANOVA are listed on the left. n is the number of accessions that displayed that trait. F is the F value from the type III sum of squares ANOVA for each factor and P is the estimated probability of obtaining this F value under the null hypothesis. Significant P values are in italic. Each experiment was repeated four times with on average 14 plants per accession per treatment per experiment.

glucosinolate accumulation in control conditions *vs.* MeJa treatment (see MATERIALS AND METHODS for complete experimental design). This identified one to five QTL regulating accumulation of specific glucosinolates under control and/or MeJa-treated conditions (Table 2). The QTL had varying significance levels and trait impacts (Table 2).

We identified QTL near *AthGAPAB* and *nga106* that specifically control the accumulation of total indole glucosinolates after MeJa treatment (Figure 4). These QTL are candidates for loci controlling phenotypic plasticity in total indole glucosinolate accumulation in response to MeJa. ANOVA with genetic markers showed that the *AthGAPAB* and *nga106* QTL significantly influence the differential MeJa regulation of total indole glucosinolate accumulation among the L*er* × Col RI lines (see Table 2 and MATERIALS AND METHODS for a complete description of the statistical model). The difference in total indole glucosinolate induction occurs because L*er* alleles at both QTL show greater MeJa induction in comparison to the Col-0 alleles (Figure 5). This agrees with the previous observation that total indole glucosinolate levels undergo a greater MeJa induction in L*er* than in Col-0 (Figure 3D). The *AthCDPK9* QTL had a significant impact on the difference between MeJa and control conditions by affecting total indolic glucosinolate concentrations only under control conditions (Table 2 and Figure 4).

3-Hydroxypropyl glucosinolate also has a number of QTL with differential MeJa impacts (Table 2 and Figure 6). Most of these QTL regulate 3-hydroxypropyl glucosinolate levels under control conditions and lose their regulatory impact after MeJa treatment (Table 2 and Figures 6 and 7). The identification of most of these QTL on a single chromosome limits the precision of these estimates due to a limited number of recombinants between the *GS-Elong* and *AthCDPK9* QTL (Figure 6).

The other major MeJa difference between L*er* and Col is in the regulation of 8-methylsulfinyloctyl glucosinolate concentration (Figures 2 and 3, A and B). Our analysis identified two QTL as possible candidates for controlling this difference, *AthUBIQUE* and *AthS0191* (Table 2). Of these, only the *AthS0191* QTL shows an allelic pattern that is consistent with the difference between the parental accessions. For this QTL, the Col-0 allele decreases the ratio of 8-methylsulfinyloctyl glucosi-

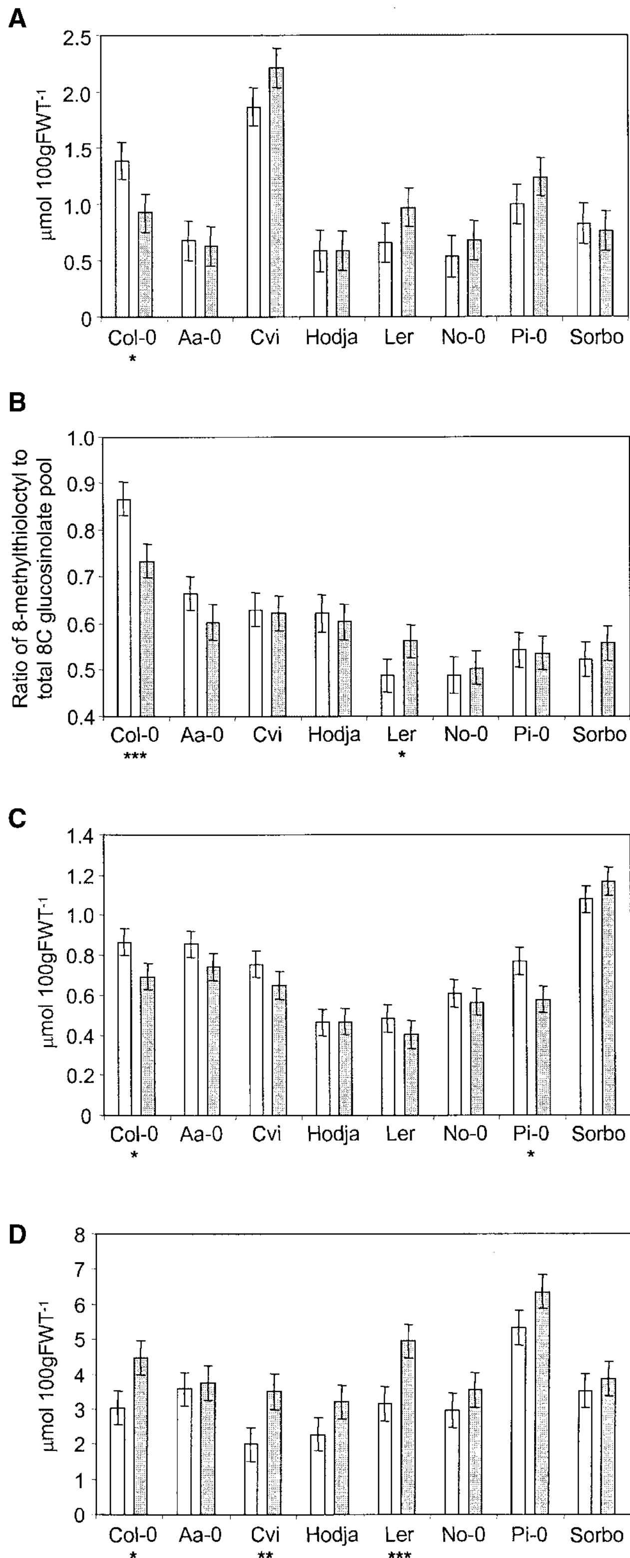

FIGURE 3.—Differential MeJa-mediated induction of various glucosinolates in eight accessions. Shaded bars represent plants treated with MeJa for 24 hr and open bars represent control plants. Asterisks below the accession represent the significance of the difference between the control and MeJa level for that glucosinolate in that accession as determined by two-tailed *t*-tests with Bonferroni-adjusted *P* values; (*) $P < 0.01$; (**) $P < 0.001$; (***) $P < 0.0001$. The absence of an asterisk indicates that the difference in control and MeJa levels is not significant for that glucosinolate in that accession. (A) 8-Methylsulfinyloctyl glucosinolate concentration. (B) Ratio of 8-methylsulfinyl to the total 8C glucosinolate pool. (C) 4-Methoxyindol-3-ylmethyl glucosinolate concentration. (D) Total indolic glucosinolate concentration.

TABLE 2

QTL identified from L*er* × Col-0 RI lines and environment interactions

Trait	Marker	Chromosome	*P*	Difference (%)	× Marker	Marker	*P*
Total indole	*AthGAPAB*	III	<0.0001	25	**		
	nga106	V	0.0007	24	**		
	AthCDPK9	V	0.4143	6	*		
Indolyl-3-methyl	*F7G19*	I	0.0427	−17	—	*AthCDPK9*	0.0044
(I3M)	*AthGAPAB*	III	0.0003	24	**		
	nga106	V	0.0033	27	—		
	AthCDPK9	V	0.0449	17	—		
1-Methoxy-I3M	*AthGAPAB*	III	0.0023	33	*		
Total aliphatic	*AthGAPAB*	III	<0.0001	21	—		
	nga158	V	0.0024	−15	—		
	GS-Elong	V	0.0023	21	—		
	AthCDPK9	V	0.0068	19	—		
	DFR	V	0.0030	−15	—	*nga158*	0.0003
8-Methylsulfinyloctyl	*AthUBIQUE*	II	0.0125	18	*		
(8-MSO)	*GS-Elong*	V	0.0417	25	—		
	AthCDPK9	V	0.0017	37	—		
8-Methylthiooctyl	*AthGPA1*	II	0.0002	−26	—		
(8-MT)	*ATHCTR1*	V	0.0009	19	—		
	GS-Elong	V	<0.0001	46	*		
	ATHPHYC	V	0.0002	22	—		
8-MSO to 8-MT	*nga158*	V	<0.0001	−40	—	*AthS0191*	0.0051
	AthS0191	V	0.0165	−20	*		
3-Hydroxypropyl	*nga8*	IV	0.8776	−1	*		
	nga126	III	<0.0001	20	—	*nga8*	0.0094
	AthCDPK9	V	0.0026	−27	****		
	GS-Elong	V	0.0006	37	*		
	DFR	V	0.3401	6	***	*nga8*	0.0361
4-Methylsulfinylbutyl	*nga158*	V	<0.0001	−98	***		
4-Methylthiobutyl	*NCC1*	I	<0.0001	33	—		
	AthGAPAB	III	<0.0001	33	—		

The QTL identified for each trait from the MeJa, control, and difference data sets are listed with the marker that maximizes the LOD score for the given QTL. *P* is the *P* value testing whether the QTL alters the given trait using the combined MeJa and control data sets. Allelic difference is the difference between the L*er* and Col-0 alleles at the given marker for the given trait. This is determined by taking the least-squares means for both alleles and using the following equation (L*er* − Col-0)/L*er*. Treatment × marker is the probability that the QTL represented by the marker has differential impacts on accumulation of the given glucosinolate between control- and MeJa-treated RI lines. * <0.05; ** <0.01; *** <0.001; **** <0.0001.

nolate to the total 8C pool in response to MeJa, while the L*er* allele causes a slight but nonsignificant increase (Figure 8).

DISCUSSION

Phenotypic plasticity: Much controversy has centered on the definition of plasticity genes as loci that influence trait changes between environments, but do not alter trait means within environments (Via *et al.* 1995). Schlichting and Pigliucci (1993) argued that plasticity and trait values are independent, and hence genes that affect only plasticity (the "gene regulation" model) must exist. Alternatively, Via (1994) argued that plasticity evolves due to selection on phenotypic values expressed within particular environments, so plasticity must be controlled by loci that are expressed within these environments (the "allelic sensitivity" model). Furthermore, Via argued that it is unnecessary to hypothesize the existence of genes that affect only plasticity, because this is inconsistent with assumptions of quantitative genetic models. This controversy can be resolved by QTL mapping experiments.

Our experimental results clearly support the allelic sensitivity model. All significant QTL that influenced plasticity between the control and MeJa-treated plants also impacted the mean trait value in at least one of the two "environments." This indicates that, at least under the conditions imposed in this experiment, plasticity was controlled by loci that determine the mean trait value under specific environments. It is possible that statistical power in this experiment was insufficient to detect plasticity genes corresponding to the gene regulation model, which might control plasticity without im-

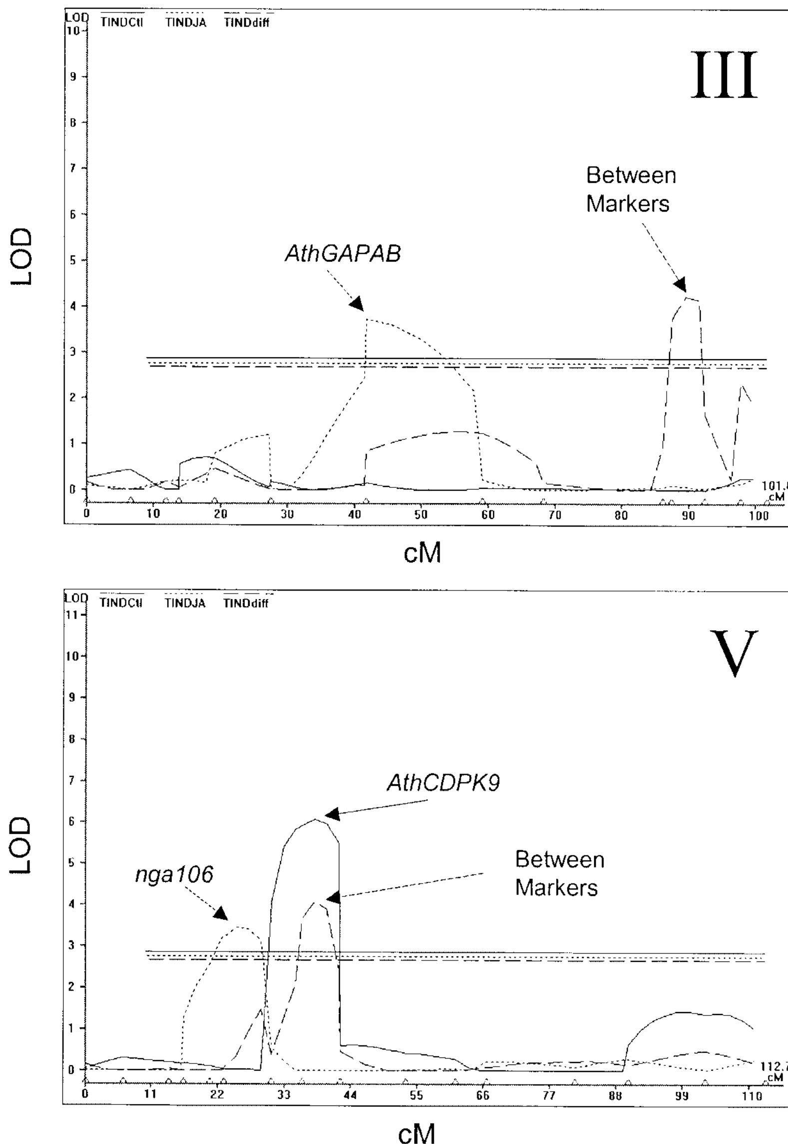

Figure 4.—QTL map for total indole glucosinolate concentration in RI lines from L*er* × Col-0. Solid lines are the QTL identified using the total indole glucosinolate concentration of plants measured under control conditions. Dotted lines are the QTL identified using the total indole glucosinolate concentration from plants measured after MeJa treatment. Dashed lines are the QTL identified using the algebraic difference between the MeJa and control measurements for each RI line. Each QTL is labeled with the marker showing the maximum LOD score. The one QTL marked "Between Markers" had no marker with a significant LOD score and was not considered in subsequent analysis. The horizontal lines represent the LOD score significant at $P = 0.05$ as determined from 500 permutation tests. For the control data the significant LOD score is 2.87, for the MeJa data the significant LOD score is 2.76, and for the difference the significant LOD score is 2.68.

pacting mean trait values. Nevertheless, experimental support for the allelic sensitivity model is clear: plasticity is controlled by the same QTL that influence trait variation within particular environments.

Multiple MeJa pathways for glucosinolate regulation: Glucosinolate responses to MeJa treatment in L*er* and Col-0 display a number of differences. This includes glucosinolate in which L*er* is more MeJa responsive than Col-0 (total indole glucosinolate, Figure 3D), Col-0 is more sensitive than L*er* (4-methoxyindol-3-ylmethyl glucosinolate, Figure 3, A and C), and the two accessions have opposite MeJa responses (ratio of 8-methylsulfinyloctyl to the total 8C glucosinolate pool, Figure 3B). Even the indole glucosinolates exhibited differential MeJa regulation patterns among the accessions (Figure 3, C and D). This variety of differences between L*er* and Col-0 suggests that MeJa regulates glucosinolate accumulation by multiple pathways. Further, because some accessions have no MeJa-mediated glucosinolate responses, a number of these pathways appear to contain natural knockouts (Figure 3 and Table 1). Because this set of genotypes was preselected for nonresponsiveness, it cannot be used to estimate the frequency of defective MeJa responses in Arabidopsis (see materials and methods).

In agreement with the accession analysis, the QTL study identified two different pathways for MeJa regulation of glucosinolates. The first pathway leads to larger induction of total indole glucosinolates in L*er* when compared to Col-0 (Figure 3). This is predominantly controlled by the MeJa-specific *AthGAPAB* QTL with two smaller QTL near *nga106* and *AthCDPK9* (Table 2 and Figures 4 and 5). 3-Hydroxypropyl glucosinolate also relies on the *nga106* and *AthCDPK9* QTL, suggesting that it shares MeJa signal transduction pathway elements with total indole glucosinolate induction (Table 2 and Figures 6 and 7). The *DFR* and *nga8* QTL indicate that there are also some unique aspects to MeJa induction of 3-hydroxypropyl glucosinolate. The second pathway

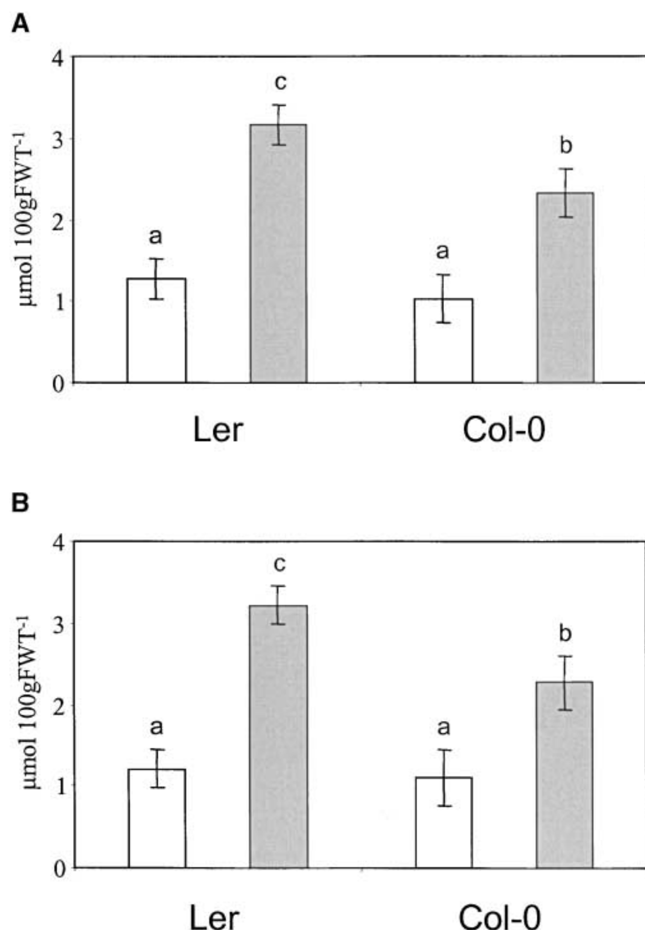

Figure 5.—The effect of two QTL that differentially control total indole glucosinolate levels in response to MeJa. Shaded bars represent the least-squares means for total indole glucosinolate level for the given allele in MeJa-treated plants. Open bars represent the least-squares means for total indole glucosinolate concentration for the given allele in control plants. Bars with different letters above them have least-squares means that are significantly different at the $P = 0.05$ level after Bonferroni adjustment. (A) QTL represented by *AthGAPAB*. (B) QTL represented by *nga106*.

controls MeJa regulation of 8-methylsulfinyloctyl glucosinolate. Two QTL, *AthUBIQUE* and *AthSO191*, neither of which control indolic glucosinolate levels, may be involved in this difference (Table 2). Thus, genetic differences among the accessions indicate that several pathways regulate these different glucosinolate responses.

Genetic variation in gene regulation: Several different MeJa signal transduction pathways regulating glucosinolate accumulation display significant natural genetic variation. This suggests that biologically important variation in gene regulation may exist among accessions. The significance of this quickly amplifies when one considers that we tested the effects of a single simple hormone treatment upon a biosynthetic pathway that probably involves <50 genes. In comparison, an Arabidopsis experiment by Maleck and co-workers tested the expression of 7000 genes under different systemic acquired resistance conditions and identified 413 genes with differential expression of 2.5-fold or more (Maleck *et al.* 2000). If this experiment with its complex treatment inputs involving SA, MeJa, and ethylene and its large number of test variables were repeated in eight accessions, the variability uncovered could be enormous. While this complicates the extrapolation of results from a genotype to an entire species, this high level of variability may enable grouping of genes into specific transcriptional clusters. It remains to be seen if the high level of regulatory variation in our study is representative of the species as a whole, or is specific to the glucosinolate pathway.

***Cis* (promoter) or *trans* (signal transduction) regulatory evolution:** A major question in the evolution of gene expression is whether variation in signal transduction typically occurs in the promoter of downstream response elements or in the upstream signal transduction pathway (Via *et al.* 1995). *Trans*-acting variation in the upstream signal transduction pathway could have broad effects upon the organism by altering regulation of a large number of genes. In contrast, mutations in the promoters of the downstream genes can generate differential regulation with less of a potential for broad impacts. Natural variants in major developmental and ecological response pathways have been identified as lesions in both upstream receptors and downstream promoters (Doebley *et al.* 1997; Carroll 2000; Grenier and Carroll 2000; El-Assal *et al.* 2001; Maloof *et al.* 2001). The observation that tryptophan-derived indole glucosinolates and the methionine-derived 3-hydroxypropyl glucosinolate share MeJa and control QTL suggests that these lesions are signal transducers rather than individual promoters. This is because tryptophan-derived indole glucosinolates and methionine-derived 3-hydroxypropyl glucosinolate are produced by two completely different biosynthetic pathways that have not been shown to share any enzymes. Thus, coregulation of their accumulation probably does not occur by metabolic mechanisms and instead appears to require the action of regulatory proteins to coordinately control the two different pathways. Identifying and characterizing the genes underlying these QTL will help to differentiate between these models.

Chromosome V linkage: Two QTL with opposite effects on most glucosinolate concentrations are tightly linked on the top of chromosome V, *AthCDPK9* and *nga106/GS-Elong* (Table 2, Figures 4 and 6). The *AthCDPK9* QTL regulates glucosinolate concentration under control conditions whereas the *nga106/GS-Elong* QTL regulated glucosinolate concentration after MeJa treatment (Table 2, Figures 4 and 6). Unfortunately, the tight linkage means that our RI population has only eight recombinants between these markers. This may bias the estimate of their separate impacts and obscure their differential MeJa effects. Fine-scale mapping using a

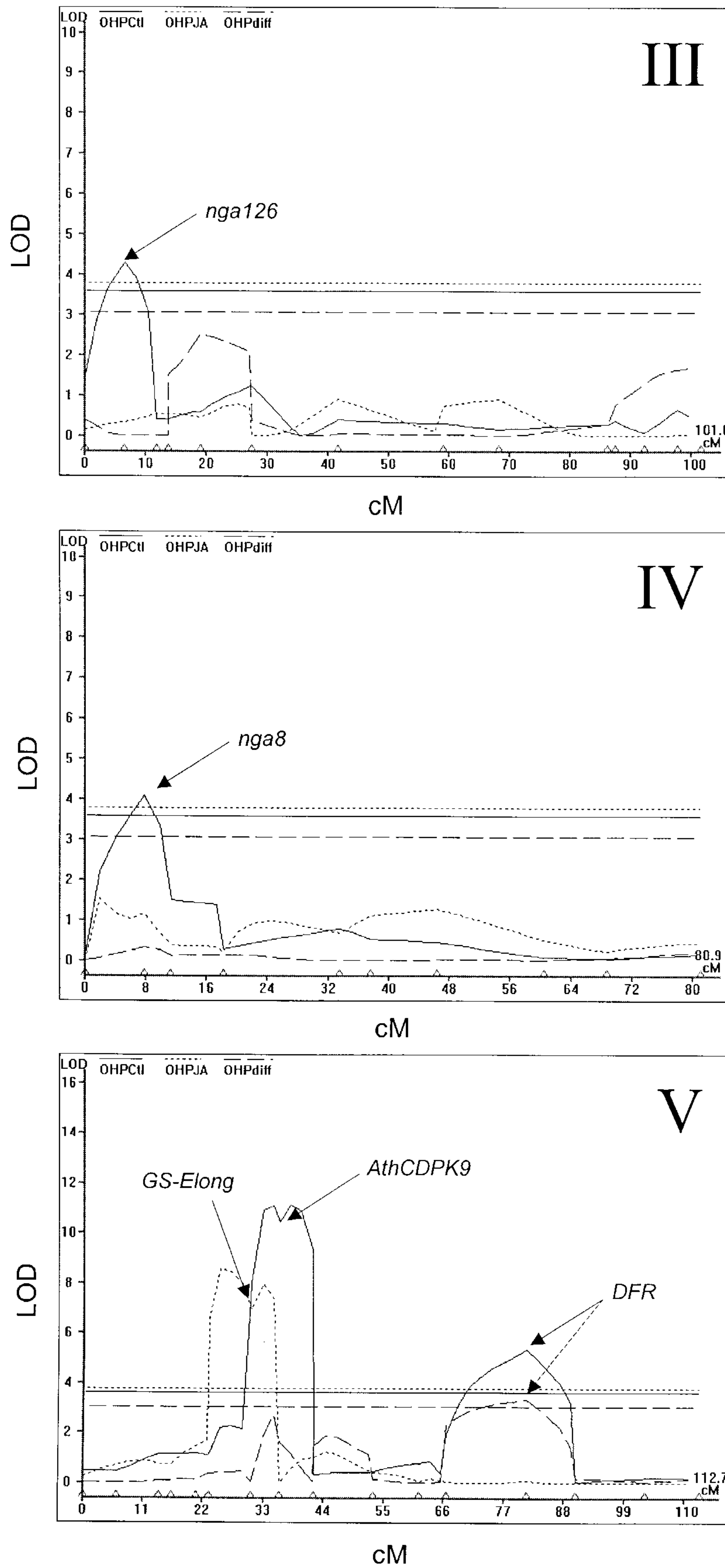

Figure 6.—QTL map for 3-hydroxypropyl glucosinolate concentration in RI lines from L*er* × Col-0. Solid lines are QTL identified using the 3-hydroxypropyl glucosinolate concentration for each RI line determined from plants measured under control conditions. Dotted lines are QTL identified using the 3-hydroxypropyl glucosinolate concentration after MeJa treatment. Dashed lines are QTL identified using the algebraic difference between the MeJa and control measurements for each RI line. Each QTL is labeled with the marker showing the maximum LOD score. The horizontal lines represent the LOD score significant at $P = 0.05$ as determined from 500 permutation tests. For the control data the significant LOD score is 3.60, for the MeJa data the significant LOD score is 3.79, and for the difference the significant LOD score is 3.28.

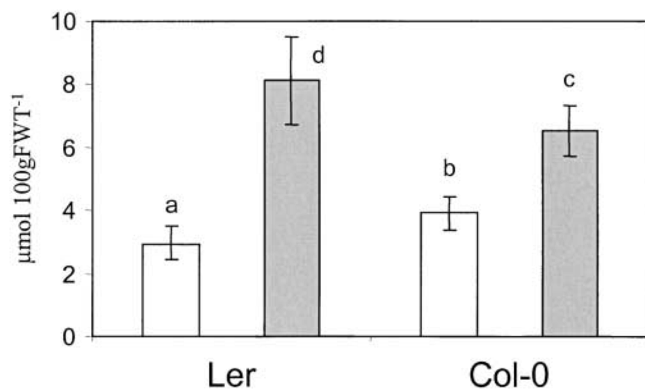

FIGURE 7.—The *DFR* QTL has differential MeJa effects on 3-hydroxypropyl glucosinolate levels. Shaded bars represent the least-squares means for the 3-hydroxypropyl glucosinolate level for the given allele in MeJa-treated plants. Open bars represent the least-squares means for the 3-hydroxypropyl glucosinolate concentration for the given allele in control-treated plants. Bars with different letters above them have least-squares means that are significantly different at the $P = 0.05$ level after Bonferroni adjustment.

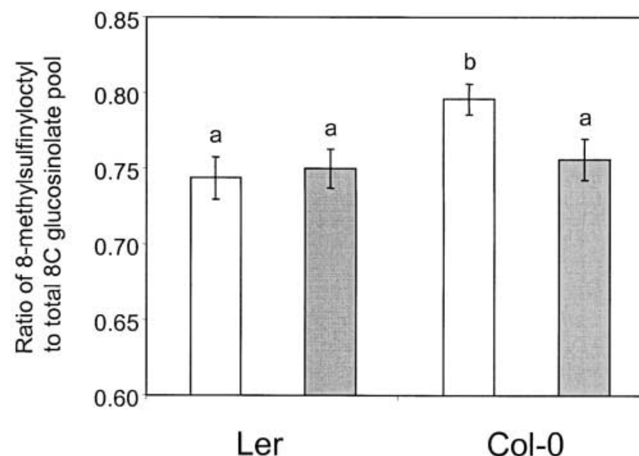

FIGURE 8.—The *ATHS0191* QTL has differential MeJa effects upon 8-methylsulfinyloctyl glucosinolate levels. Shaded bars represent the least-squares means for the ratio of 8-methylsulfinyloctyl glucosinolate to the total 8C glucosinolate pool for the given allele in MeJa-treated plants. Open bars represent the least-squares means for 8-methylsulfinyloctyl glucosinolate relative to the total 8C glucosinolate pool for the given allele in control plants. Bars with different letters above them have least-squares means that are significantly different at the $P = 0.05$ level after Bonferroni adjustment.

large number of recombination events on the top of chromosome V is required to separate their effects.

Differential and combinational glucosinolate regulation by MeJa and SA: Treatment of L*er* with either MeJa or SA alone differentially regulates the accumulation of various glucosinolates (Figure 2). MeJa induces indol-3-ylmethyl glucosinolate while SA induces 4-methoxyindol-3-ylmethyl glucosinolate (Figure 2). Further, the combination of both MeJa and SA counteracts the effect of either treatment alone (Figure 2). This negative interaction between MeJa and SA has been previously observed in Arabidopsis and other species (PEÑA-CORTE *et al.* 1993; GUPTA *et al.* 2000). In addition to a negative interaction, MeJa and SA positively interact to induce 8-methylsulfinyloctyl glucosinolate accumulation in *Ler* (Figure 2). Interestingly, this glucosinolate is repressed by MeJa treatment alone in the Col-0 accession (Figure 3A). A detailed analysis of how 8-methylsulfinyloctyl glucosinolate levels respond to combined MeJa and SA treatment in Col-0 could illuminate how these two pathways interact.

Is Cvi deficient in global MeJa sensitivity? Cvi is known to have reduced MeJa sensitivity, which elevates ozone-induced hypersensitive cell death in comparison to Col-0 (RAO *et al.* 2000). This decreased MeJa responsiveness also lowers MeJa induction of AtVSP mRNA (RAO *et al.* 2000). In agreement, Cvi is less MeJa responsive than Col-0 to 8-methylsulfinyloctyl and 4-methoxyindol-3-ylmethyl glucosinolate (Figure 3, A–C). However, Cvi induces total indole glucosinolate accumulation more than Col-0 does in response to MeJa (Figure 3D). Because Cvi glucosinolates have both elevated and reduced MeJa sensitivity in comparison to Col-0, it is not possible to classify Cvi as having a general MeJa perception deficiency. Instead, any fault is probably in a branch downstream of MeJa perception, or multiple pathways independently detect MeJa.

Future work: Variability in glucosinolate responses to MeJa generates a useful system for studying MeJa signal transduction as well as the genetics of phenotypic plasticity. Identification of further QTL in other crosses will lead to a better understanding of how the different MeJa regulatory pathways interact. Further, cloning and characterization of the genes underlying QTL that control the differential MeJa regulation will generate a detailed understanding of the molecular and biochemical basis for MeJa signal transduction and how this alters phenotypic plasticity in more complex environments. Experiments are underway to address both of these issues and to identify molecular markers that mirror the different glucosinolate responses to MeJa.

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